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THE PATHOLOGY AND PATHOGENESIS OF CLINICAL ACUTE NEPHRITIS *

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In this publication clinical acute nephritis includes all cases of acute renal disease that exhibit a definite impairment of renal function indicated by retention of nitrogenous products, decreased ability to excrete phenolsulphonephthalein, inability to form a concentrated urine, loss of large amounts of protein in the urine, bleeding from the parenchyma of the kidney and severe oliguria or anuria. All forms of benign albuminuria, including febrile, are excluded since these show only trivial alterations in renal structure and function. Acute pyelonephritis and acute obstructive lesions of the urinary tract are also omitted from this discussion.

In the literature the various anatomical types of acute nephritis are not often clearly distinguished and for this reason it is difficult to compare the observations of different investigators. Many writers discuss acute nephritis as a clinical entity and assume that the underlying pathological process in the entire group varies only in intensity and not in character. Other authors distinguish nephritis and nephrosis and interpret nephrosis as tubular disease.

The 110 cases of acute nephritis which were available for study were subdivided into groups in accordance with the structural changes in the kidneys.

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I. Acute Glomerulonephritis	
(A) Acute proliferative glomerulonephritis	
(*a*) Uncomplicated type	31 cases
(*b*) Associated with another disease	20 "
(B) Thrombosis of glomerular capillaries	1 "
(C) Exudative type	3 "
(D) Thrombosis of afferent arterioles	2 "
(E) Embolic type with uremia	
(*a*) With endocarditis	2 "
(*b*) Without endocarditis	5 "
(F) Hemorrhagic type with tubular obstruction	11 "
(G) Acute lipoid nephrosis	4 "
Total glomerulonephritis	79 cases
II. Vascular Lesions	
(A) Thrombosis of the renal arteries	1 case
(B) Widespread thrombosis of small arteries	1 "
(C) Polyarteritis nodosa	1 "
III. Interstitial Lesions	
(A) Acute interstitial nephritis	1 "
(B) Acute lymphatic leukemia	1 "
IV. Tubular Diseases of the Kidneys	
(A) Pure tubular degeneration	
(*a*) Mercuric chloride poisoning	7 cases
(*b*) Other types	2 "
(B) Obstructive tubular disease from blood transfusion	3 "
V. Extrarenal Uremia	
(A) With renal injury	8 "
(B) Without renal injury	7 "

I. Acute Glomerulonephritis

(*A*) *Acute Proliferative Glomerulonephritis* (*Table I, Types 1a and 1b*)

This variety of acute nephritis is characterized histologically by increase in the number and size of the endothelial cells of the glomerular capillaries with subsequent partial or complete obstruc-

tion of the capillary lumens. It is of more interest than other forms since more clinical cases belong in this group and nearly all chronic glomerulonephritis originates from this type.

In a previous publication (Bell, 1936) the subclinical forms of acute proliferative glomerulonephritis were described. It was pointed out that various acute infections, notably subacute bacterial endocarditis and puerperal sepsis, cause a more or less marked increase in the number and size of the glomerular endothelial cells. There is no sharp histological distinction between subclinical and clinical glomerulonephritis, but, in general, clinical symptoms appear as soon as hyaline fibers have formed in the capillaries. In this discussion a case is considered clinical when the glomerular lesions are severe, and in most instances clinical evidence of acute nephritis was also recorded.

There were 31 fairly typical examples of clinical acute proliferative glomerulonephritis (Table I, Type 1*a*), and there were 20 cases (Type 1*b*) in which the symptoms of nephritis were overshadowed by some major illness. The structural changes in the kidneys were identical in the two subgroups.

The kidneys are usually enlarged; in 31 of 37 adults the combined weight was over 300 gm., and in 13 cases over 500 gm. On section the surfaces are invariably pale and cloudy. On the basis of the macroscopic appearance alone a diagnosis of acute nephritis cannot be made with certainty since many kidneys with simple cloudy swelling present similar features.

Microscopically under low magnification the glomeruli appear very cellular and few or no erythrocytes are to be seen. There is obviously a great increase in the number of nuclei and the capillaries are occluded with nucleated cells. It is noteworthy that the glomeruli are not always greatly enlarged; in fully one-third of the cases there is only a moderate increase in their diameters. There is usually a high degree of endothelial proliferation in the capillaries of a great majority of the glomeruli, but there are always a few glomeruli with only a slight endothelial increase. In some of the cases where death was due to edema of the lungs (Nos. 20, 23, 26), at an early stage of the disease the endothelial proliferation is less pronounced than in those where death was due to uremia, but there is no close correlation between the duration of symptoms and the degree of endothelial increase. Apparently the process proceeds more rapidly in

some instances than in others. When the glomerular tufts are compressed by large epithelial crescents (Nos. 27, 28, 29, 50), the endothelial proliferation is much less pronounced.

Hyaline fibers can always be seen in sections stained by the Mallory-Heidenhain stain (azocarmine) except in tufts compressed by large epithelial crescents. These fibers are at first few in number and delicate, but later they usually become more numerous and coarser. They are apparently derived from the capillary basement membrane, especially the part forming the deep surface of the capillary wall. This feature will be discussed more fully later.

The capillary basement membrane does not often show a uniform diffuse thickening, as seen in eclampsia and in many cases of lipoid nephrosis. When the membrane is thickened it is usually split into two or three layers (No. 7) and does not appear as a single heavy band, as in the diseases just mentioned. The changes in the basement membrane will be described in more detail in the discussion of pathogenesis.

Polymorphonuclear leukocytes are found in increased numbers in 34 of the 51 cases. They were searched for only in hematoxylin-eosin preparations; the oxidase reaction would probably have demonstrated an increase in every instance. The prominence of the leukocytes is indicated in the table by numerals, "0" being the number found in normal glomeruli. When the leukocytes were the chief cause of the capillary obstruction (Grade 4) the kidney was classified as an exudative type of nephritis (Type 3). There is evidently an exudative feature in most of the cases of the proliferative type, but obstruction is due almost entirely to endothelial cells.

There is thrombosis of occasional afferent glomerular arterioles in 11 of the 51 cases. When this was the outstanding alteration responsible for renal insufficiency the kidneys were classified in a special group (Type 4).

Epithelial crescents are present in 18 of the 51 cases, and sometimes they are more important than endothelial proliferation in causing obstruction of the glomerular circulation (Nos. 27, 28, 29, 50). Large crescents compress the glomerular tufts to such a degree that no blood can pass through the capillaries. Nephritis in which epithelial crescents are unusually prominent is sometimes called "extracapillary" glomerulonephritis, but the extracapillary lesions

are so intermingled with the intracapillary proliferative forms that they hardly constitute a distinct histological type of nephritis.

It is clear that there are relatively few cases of pure endothelial proliferation; there are commonly some polymorphonuclear leukocytes, epithelial crescents or thrombosed arterioles. The changes in the capillary basement membrane will be described presently.

Histogenesis of the Glomerular Lesions: In a previous publication the subclinical stages of glomerulonephritis have been described. In infectious diseases, especially bacterial endocarditis and puerperal sepsis, there is often a rather marked endothelial increase which differs from clinical acute glomerulonephritis only in intensity and in the absence of hyaline fibers. There are gradual transitions between the subclinical and the clinical stages. Apparently symptoms and signs of nephritis usually do not appear until glomerular obstruction is pronounced.

The less advanced lesions in clinical cases correspond entirely to those of the subclinical stage previously described. The capillaries are partially filled with mononuclear cells attached to the basement membrane. These cells have been interpreted by nearly all investigators as of endothelial origin. Recently, however, MacCallum has sponsored the view that they arise from connective tissue cells between the capillaries. MacCallum's theory is based largely on his observation that the capillary basement membrane surrounds the minute lobules of the glomerulus but does not form a complete investment for each capillary. However, in a normal glomerulus, appropriately fixed and stained, the basement membrane is easily seen as a complete layer around each capillary; it is just as prominent on the inner walls of capillaries within the lobule as it is at the surface of the lobule. In the subclinical stages of glomerulonephritis the basement membranes within the lobule are also readily seen and there is no doubt that the cells in question lie within the capillaries (see *Am. J. Path.*, 1936, **12**, 801, Figures 3, 5 and 6). However, after a glomerulonephritis is established the layer of basement membrane on the deep surface of the capillary splits into fragments, which form hyaline fibers, and a continuous basement membrane is no longer seen except at the surface of the lobule (see *Am. J. Path.*, 1936, **12**, Plate 132, Figs. 7 and 8). A study limited to well developed examples of glomerulonephritis might lead one to the false conclusion that the basement membrane is absent within the lobule and that

intercapillary fibroblasts give rise to the cells and fibers that obstruct the capillaries.

An early stage of clinical glomerulonephritis is shown in Figure 1. The small lobule shown in the drawing is only a little more advanced than the subclinical stage (compare *Am. J. Path.*, 1936, **12**, Plate 131, Fig. 6). The capillaries are well filled with large endothelial cells and only an occasional erythrocyte is to be seen. The basement membrane of the outer wall of the capillaries is largely intact but the layer forming the inner capillary wall is no longer even and continuous, as it is in the normal and the subclinical stages. It shows several interruptions and there are a few delicate blue stained fibrils apparently branching off from this layer and penetrating the cytoplasmic mass. This is an early stage in the formation of hyaline fibers and they appear to have been split off from the inner basement membrane.

In Figure 2 a later stage is illustrated. The basement membrane of the outer surface of the capillaries is intact but the inner layers are split into numerous hyaline fibers. The individual capillaries can no longer be distinguished. Unless the splitting of the inner basement membrane is followed from its earliest stages it may appear that there is no basement membrane covering the deep surface of the capillaries and that the hyaline fibers are derived from intercapillary fibroblasts. But when successive stages of the process are studied it is readily seen that the hyaline fibers are split off from the inner basement membranes. There are no intercapillary cells in these small peripheral lobules, except occasional epithelial cells that extend inwards from the surface layer.

When the reaction has progressed to the stage shown in Figure 2 the inner walls of the capillaries can no longer be identified, since the capillaries have become fused on their deep surfaces. The central portion of the lobule is now composed of endothelial cells and hyaline fibers. In the lobule shown in Figure 2 all the capillary lumens have been obliterated, but often portions of the lumens persist at the periphery. This appearance may give the impression of an intercapillary accumulation of cells and fibers, but it is actually due to fusion of the adjacent capillaries and splitting of the inner basement membranes.

When portions of the capillary lumens at the periphery of the lobule persist and enlarge the fibers are pushed toward the center

of the lobule where they fuse into a homogeneous mass which gives the impression of intercapillary material. In some cases of chronic glomerulonephritis the central hyaline masses in the lobules are conspicuous and have been interpreted as "intercapillary glomerulonephritis." However, if one follows glomerular lesions from their earliest stages it becomes clear that all so-called intercapillary lesions are due to splitting and duplication of the inner basement membranes and subsequent compression of them in the center of the lobule. This feature will be described more fully in a subsequent paper.

When the capillaries of a glomerulus are completely filled with endothelial cells and hyaline fibers the glomerulus soon undergoes hyaline degeneration, if the patient survives, but this does not occur in the acute stage. When the capillaries are not completely closed circulation continues in the periphery of each lobule.

In acute proliferative glomerulonephritis terminating in uremia the glomerular lesions have commonly progressed no farther than the stage shown in Figure 2. In a subsequent paper these lesions will be followed into the chronic stage. It will be shown that glomeruli with completely closed capillaries soon become hyaline. When the capillaries are incompletely closed the force of the blood flow seems to open them to some extent, the hyaline fibers and cells being pushed toward the walls of the capillaries. Nearly all persistent glomeruli in chronic cases show thick basement membranes.

It is probable that lesions, such as shown in Figure 1, may terminate in recovery. We do not know the glomerular structure in instances of recovery, but presumably the alterations are much less intense than in those of death from uremia.

(*B*) *Thrombosis of Glomerular Capillaries* (*Table I, Type 2*)

This patient (No. 52) presented the clinical features of septicemia rather than of nephritis although she had albuminuria and hematuria. The glomeruli show no exudative or proliferative changes but a large proportion of the glomerular capillaries is occluded by hyaline thrombi (Fig. 3). The thrombi are similar to the acute thromboses of bacterial endocarditis, but they do not have a focal distribution. There was no endocarditis in this case. In Case No. 31 there are also many thrombosed capillaries but the chief lesion is proliferative glomerulitis.

(*C*) *Exudative Type* (*Table I, Type 3*)

This form of nephritis is usually associated with a severe staphylococcic infection which may overshadow the renal symptoms. In 1 case (No. 55) renal insufficiency was demonstrated, and in another (No. 54) a severe oliguria was present. The glomerular capillaries are all distended with polymorphonuclear leukocytes, and these cells are also found in large numbers in the tubules. This form of glomerulitis presumably does not pass over into a chronic type. Staphylococci are believed to be the causative agents.

(*D*) *Thrombosis of Afferent Arterioles* (*Table I, Type 4*)

One of the patients of this group (No. 56) was a young infant with congenital syphilis, complicated in the terminal stages by pneumonia and pneumococcic peritonitis; the other patient (No. 57) was diagnosed clinically as suffering from acute nephritis and died in uremia. In both cases a large majority of the afferent glomerular arterioles are occluded by fresh hyaline thrombi. Some of the glomeruli are infarcted but they show no other striking changes. Juhel-Rénoy, in 1886, reported a case of this type in a girl 16 years of age. The patient developed anuria 2 days after the appearance of a scarlet fever eruption, which persisted until death 5 days later. Extensive thrombosis of glomerular arterioles was found at postmortem.

(*E*) *Embolic Type With Uremia* (*Table I, Type 5*)

It is well known that bacterial endocarditis may terminate in uremia. Usually in such cases a diffuse proliferative glomerulonephritis is found at postmortem, but sometimes one finds numerous massive lesions of the embolic type which have blocked nearly all of the glomeruli (Fig. 4). Lesions of embolic type may occur in the absence of endocarditis; in fact endocarditis was present in only 2 of the 7 cases of this type, and we have 2 cases of embolic glomerulonephritis in which endocarditis was present only on the valves of the right heart. Patients Nos. 58 and 59 were diagnosed clinically as suffering from endocarditis and it was recognized that the former died of uremia. The other 5 cases were interpreted clinically as acute glomerulonephritis. In a previous publication (1932) I have described the structure of these so-called embolic lesions; they are

embolic in the sense that they are caused by the lodgment of bacteria but not in the sense that they cause infarction of parts of the glomerulus. There are two types of embolic lesions: the acute thrombotic form and the fibrous lesion. The thrombotic lesions are merely capillary thromboses, usually with necrosis of some of the capillary loops. A few thrombosed capillaries are frequently seen in the proliferative type of nephritis and in 3 cases (Nos. 31, 51 and 52) they were present in enormous numbers. The fibrous lesions are caused by focal splitting and thickening of capillary basement membranes. Contrary to the common belief the fibrous lesions do not develop from the thrombotic ones. In the cases listed (Nos. 58 to 64) the lesions are numerous and large, and both thrombotic and fibrous types are present. Probably these lesions are produced by bacteria circulating in the blood stream, which lodge in the glomerular capillaries.

(*F*) *Hemorrhagic Type With Tubular Obstruction* (*Table I, Type 6*)

This form of nephritis is characterized clinically by a severe infection of some kind and a pronounced hematuria. Often there is a bacteriemia. In 6 of the 7 cases in which blood urea nitrogen was determined it was found markedly increased. One patient had anuria.

The glomerular capillaries in these kidneys show only slight to moderate endothelial increase and there is evidently very little obstruction of their lumens. A great many of the capsular spaces and tubules are, however, distended with blood (Fig. 5). The blood evidently escapes from minute ruptures in the glomerular capillaries. In many of the tubules the red cells appear as compact masses, often with threads of fibrin between them; in other tubules many of the erythrocytes are laked, and amorphous masses of hemoglobin are seen. In the collecting tubules especially there are numerous loose casts composed of hemoglobin, erythrocytes and albumin (Fig. 6). The cortical tubules are uniformly dilated, presumably because of the numerous casts. The tubular epithelium often shows hyaline granular degeneration but very few cells are necrotic. The tubular obstruction is not unlike that seen in the transfusion kidney to be described presently, except that the obstruction is usually due to erythrocytes rather than to hemoglobin.

It appears that bacterial toxins injure the glomerular capillaries causing them to rupture and liberate large quantities of blood into

TABLE I

Group 1. Acute Glomerulonephritis

Type 1 a. Proliferative Type — Uncomplicated by Other Diseases

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data	Chronic passive congestion of liver
		yrs.							*mg. per 100 cc.*							*gm.*		
1	13–140	12	F	3 mos.	3	0	4	?	?	3	1	0	1–	0	0	Normal	A severe cold followed by pneumonia. Pneumococcic peritonitis	1
2	15–65	55	M	6 wks.	2	+	2	?	?	3	1	0	1–	0	0	Normal	No preceding infection	?
3	15–230	34	M	?	?	?	0	?	?	2	1	0	1–	0	0	490	No history	?
4	17–176	66	F	1 mo.	3	0	1	?	72 (2 wks.)	3	1	0	1	1–	0	300	A cold followed by bronchitis	0
5	19–152	12	M	5 wks.	3	0	2	132/94	?	3	2	0	1–	0	0	245	Followed smallpox	1
6	21–108	51	M	1+ mo.	?	?	2	228/110	?	3	3	0	0	1	0	630	No preceding infection	0
7	22–468	35	M	2 mos.	?	+	2	100/ ?	?	3	1	3	1–	0	2	365	Followed pneumonia. Empyema. Multilayered basement membrane	2

8	22–553	7	F	4 days	?	?	3	?	?	3	1	0	0	0	0	150	Scarlet fever 2 wks. before edema. *Strept. viridans* bacteriemia	?
9	23–33	11	F	1 wk.	3	+	2	?	?	3	1	0	2	0	0	230	No preceding infection. Blood in the tubules	?
10	23–298	36	F	2 mos.	2	–	1	190/124	?	3	2	0	1	0	1–	460	Followed sore throat	1
11	23–419	16	F	3 wks.	2	0	0	154/92	36 (3 days)	3	3	0	0	0	0	295	Began 3 weeks after labor	3
12	24–540	2	M	1 wk.	?	?	1	110/ ?	?	2	1	0	2	0	1–	250	Onset 3 weeks after scarlet fever. Pyuria, later anuria. Blood, pus and casts in tubules	?
13	26–18	60	M	?	?	?	0	?	?	3	1	0	1	0	0	390	No preceding infection	0
14	28–189	61	M	6 wks.	3	+	0	150/75	145 (3 wks.)	3	2	0	2	0	3	320	Followed a severe sore throat	0
15	28–1129	5	F	6 days	3	+	2	?	?	3	1	0	0	1	0	159	No preceding infection. Severe oliguria	3
16	29–253	56	M	10 days	4	+	3	130/68	75 (2 days)	2	1	0	0	0	1–	620	Influenza 3 wks. before onset. Blood in tubules	0

TABLE I. GROUP 1. TYPE 1 *a*—*Continued*

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data	Chronic passive congestion of liver
		yrs.							*mg. per 100 cc.*							*gm.*		
17	29–1799	62	M	1 mo.	4	0	1	190/105	190 (8 days)	2	2	0	0	0	1	550	Influenza 2 wks. before onset	1–
18	30–526	61	M	3 wks.	3	0	0	?	?	3	1	0	2	0	0	550	Onset 1 wk. after a sore throat. Oliguria. Anuria	1
19	30–919	68	F	1+ mo.	?	?	0	190/80	?	3	1	0	2	1–	2	385	A cold 3 mos. before death. Pyuria	1
20	32–1642	7	F	6 days	?	?	1	?	?	2	1	0	1–	0	0	148	Sore throat 3 wks. before death. Death from edema of lungs	0
21	32–1706	7	M	3 days	4	0	1	?	?	3	1	0	1	0	0	150	Developed impetigo contagiosa 3 wks. before death. Severe edema of lungs	2
22	33–302	51	M	16 days	3	0	3	164/80	100 (4 days)	3	1	0	1	0	0	420	Sore throat 3 wks. before death	1
23	33–469	7	M	?	?	+	0	?	?	2	1	0	1–	0	0	?	Scarlet fever 6 wks. before death. Death from edema of lungs	1

24	33–1598	14	F	9 days	?	?	1–	160/115	?	3	1	0	2	0	0	170	Onset with sore throat and bronchitis	1
25	35–638	35	M	2 wks.	4	0	3	146/90	35 (1 hr.)	3	3	0	1	0	0	460	No preceding infection. Death from edema of larynx and lungs	?
26	35–793	35	F	6 wks.	2	?	2	?	70	2	1	3	1	0	1	?	Edema of lungs	?
27	18–62	27	M	5 wks.	3	0	1	?	?	1	0	0	3	2	4	290 (one)	Nephrectomy followed by suppuration of wound. Pyuria	0
28	30–602	1	M	5 wks.	4	0	0	?	?	1	0	0	0	0	4	200	Pharyngitis. Otitis media	0
29	32–1434	32	M	5 wks.	?	?	3	180/110	NPN 159 (5 days)	2	0	2	0	0	4	535	Cold followed by bronchitis 9 wks. before death	0
30	35–752	44	F	10 wks.	4	0	1	150/60	?	3	3	2	2	0	0	270	Hydrothorax (3). Ascites. PSP 30% (2 wks.). Ruptured aneurysm of mesenteric artery	0
31	24–288	40	F	1 mo.	2	0	1	180/110	32 (3 wks.)	3	1	0	0	0	1	490	Numerous thromboses in glomerular capillaries	0

TABLE I. GROUP 1 — *Continued*

Type 1 b. Proliferative Type — Associated With Other Diseases

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data
		yrs.							*mg. per 100 cc.*							*gm.*	
32	17–202	25	M	?	3	?	4	140/80	?	3	3	0	1	0	0	515	Bacterial endocarditis
33	22–581	19	F	6 wks.	?	?	2	170/ ?	?	3	1	0	1	1 –	0	350	Puerperal sepsis. *Strept. viridans* septicemia
34	18–122	30	M	?	3	0	3	?	?	2	1	0	1 –	0	0	530	Bacterial endocarditis
35	32–910	11	F	3 wks.	1 –	0	0	?	?	3	2	0	0	1 –	0	210	Lupus erythematosus
36	36–71	40	F	?	3	0	1	132/78	86 (6 days)	2	1 –	0	1	1	0	345	Organizing pneumonia
37	16–48	58	M	3 wks.	?	?	1	140/ ?	?	3	1	0	3	1	2	573	Diabetic gangrene. Arteriolosclerosis
38	20–220	55	M	?	?	?	3	?	?	3	1	0	2	0	0	397	Alcoholism. Hypertension
39	25–360	69	M	10 days	4	0	1	?	?	3	1	0	1	1 –	0	370	Began with a cold. Hypertension. Arteriolosclerosis
40	29–456	78	M	3 wks.	?	?	2	215/110	131 (1 day)	3	3	0	0	0	3	260	Hypertension. Arteriolosclerosis

40	29-430	78	M	3 wks.	?	?	2	215/110	131 (1 day)	3	3	0	0	0	3	260	Hypertension. Arteriolosclerosis	Ar-
41	32-782	60	M	?	2	0	1	265/142	34 (1 mo.)	3	3	0	1	0	1	450	Hypertension. Many capillary thrombi	
42	24-580	68	M	?	3	0	3	200/115	41 (6 wks.)	2	1	0	0	1	0	275	Hypertension	
43	32-798	62	M	3 mos.	1–	0	1	220/140	103 (1 day)	3	1	0	0	0	0	510	Hypertension with cardiac decompensation	
44	33-64	74	M	?	1–	0	3	190/75	?	3	1	0	1	0	0	710	Hypertension. Pyonephrosis	
45	31-1423	37	M	?	3	0	2	120/70	30 (3 mos.)	2	1	1	0	0	1	275	Old aortic valve defect with cardiac decompensation	
46	30-259	78	M	5 mos.	?	+	2	130/78	158 (2 days)	3	1	0	2	0	3	200	Hypertrophy of prostate. Infected surgical wound	
47	32-391	56	F	?	2	0	1	160/80	77 (1 day)	3	3	2	0	0	0	515	Cystitis. Hydronephrosis. Arteriolosclerosis	
48	32-619	13	M	3 wks.	2	0	1	120/74	78 (4 days)	3	2	0	1	0	0	400	Pulmonary tuberculosis. Began with a cold	
49	35-1181	38	F	?	1–	0	0	90/60	?	3	3	0	1	0	0	400	Hypertension with cerebral hemorrhage	
50	31-954	35	M	?	?	?	0	?	?	2	1	1	0	0	4	500	Bacterial endocarditis. No embolic lesions	

TABLE I. GROUP 1. TYPE 1 *b* — *Continued*

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data
		yrs.							*mg. per 100 cc.*							*gm.*	
51	32–255	62	M	?	?	?	0	200/100	?	2	1	0	0	0	0	441	Hypertension. Extensive thrombosis of glomerular capillaries
								Type 2. Thrombosis of Glomerular Capillaries									
52	34–711	44	F	9 days	3	+	0	128/72	14 (5 days)	0	0	0	0	0	0	460	Began with upper respiratory infection. Septicemia. Widespread thrombosis of glomerular capillaries
								Type 3. Exudative Type									
53	15–323	49	M	?	2	0	2	160/110	?	2	0	0	4	0	3	330	Aortic aneurysm
54	18–251	18	F	4 days	4	0	1–	?	?	1	0	0	4	0	0	540	Peritonsillar abscess. Hemolytic streptococcus bacteriemia. Severe oliguria
55	25–222	18	F	?	3	0	0	140/76	138 (1 wk.)	1	0	0	4	0	0	671	Pelvic abscess. Pyemia

55	25–922	18	F	?	3	0	0	140/76	138 (1 wk.)	1	0	0	4	0	0	671	Pelvic abscess. Pyemia

Type 4. Thrombosis of Afferent Arterioles

56	18–9	3.5 mo.	F	3 mos.	?	?	0	?	?	0	0	0	0	4	0	45	Congenital syphilis, pneumonia, pneumococcic peritonitis
57	24–185	13	M	5 wks.	3	0	1–	120/70	235 (2 days)	1	1–	0	1	4	0	230	No preceding infection

Type 5. Embolic Type With Uremia

58	22–554	43	M	?	1	0	1	140/60	119 (2 days)	0	0	0	0	0	2	270	Bacterial endocarditis. Widespread large embolic lesions
59	34–953	39	M	?	3	?	0	93/63	?	0	0	0	0	0	3	380	Bacterial endocarditis. Widespread large embolic lesions
60	25–945	41	M	?	2	+	1	248/102	80 (6 wks.)	1	0	1	0	0	2	355	Extensive embolic lesions. No endocarditis. Chronic bacteriemia
61	33–292	34	F	3 mos.	4	0	2	170/90	84 (1 hr.)	1	0	1	0	0	0	462	Extensive embolic lesions. No endocarditis
62	32–206	67	M	2 mos.	4	0	1	168/90	133 (2 days)	1	0	1	0	0	0	380	Extensive embolic lesions. No endocarditis

TABLE I. GROUP 1. TYPE 5 — *Continued*

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data
		yrs.							*mg. per 100 cc.*							*gm.*	
63	22–363	23	M	2 mos.	1	0	1	?	153 (8 days)	1	0	1	0	0	3	425	Extensive embolic lesions. No endocarditis
64	34–584	50	M	5 wks.	4	+	1	130/100	167 (3 days)	1	1	0	0	0	3	570	Extensive fibrous embolic lesions. No endocarditis
									Type 6. Hemorrhagic Type								
65	13–153	13	F	15 days	?	++	0	?	?	1	0	0	0	0	0	Large	Began with sore throat. Severe oliguria. Streptococcic peritonitis
66	19–23	31	F	6 wks.	?	++	0	?	?	1	0	0	1	0	0	440	Pneumococcic bacteriemia and meningitis
67	23–737	15	F	2 mos.	?	++	1–	?	?	1	0	0	1	1–	0	410	Streptococcic bacteriemia and peritonitis

68	26–209	9	F	3 wks.	?	++	1	?	58 (11 days)	1	0	0	0	0	1–	330	Followed scarlet fever. Otitis media. Streptococcic bacteriemia
69	26–515	24	M	1 mo.	?	++	0	148/60	122 (6 days)	1	0	0	1	1–	1	Very large	Acute osteomyelitis. A few embolic lesions. No endocarditis
70	31–804	55	F	2 wks.	1	++	1	?	95 (1 day)	2	0	0	0	0	2	405	Enormous hemorrhage into tubules. A few embolic lesions. No endocarditis
71	33–235	56	F	?	3	++	1	166/92	87 (12 days)	2	0	0	1	1–	0	400	Hyperthyroidism
72	33–832	25	F	4 days	?	?	0	?	85 (2 days)	0	0	0	0	0	0	500	Puerperal sepsis. Tubules full of blood
73	35–1961	50	M	1 wk.	1–	+	0	170/100	?	1	0	0	0	0	0	398	Lobar pneumonia. Tubules full of blood
74	33–288	5	M	8 days	?	++	0	?	168 (2 days)	2	1–	0	0	2	0		Sore throat. Streptococcic peritonitis. Anuria
75	33–452	22	F	1 mo.	1	++	0	128/90	28 (2 days)	2	0	0	0	0	0	525	Followed a peritonsillar abscess. Severe vomiting

TABLE I. GROUP 1 — *Continued*

Type 7. Acute Lipoid Nephrosis

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data
		yrs.							*mg. per 100 cc.*							*gm.*	
76	34–179	4	M	2 mos.	4	0	1	108/70	23.8 11.6	1	0	0	0	0	0	290	Began with conjunctivitis. Death from streptococcic peritonitis
77	35–114	58	M	4 mos.	4	0	3	140/90	15.9	0	0	2	0	0	0	270	Oliguria. Streptococcic peritonitis
78	35–654	36	M	5 wks.	4	0	3	?	?	0	0	0	0	0	0	365	NPN 38 mg. (2 wks.). Death due to accident
79	34–383	1½	M	6 wks.	4	0	3	?	?	1	0	0	0	0	0	300	Otitis media. Peritonitis. Streptococcic fatty tubules

0 = normal. The intensity of the various processes is indicated by numerals.
Under urea nitrogen the time before death is indicated.

the tubules. The blood cells become impacted in some of the tubules and actual coagulation sometimes occurs. Hemoglobin casts are rare and may be due to hemoglobinemia or to laking of the red cells in the tubules. Although the uremia accompanying this type of nephritis is due to tubular obstruction the primary injury is glomerular and for this reason the disease is classified as glomerulonephritis.

The bleeding glomeruli show no lesions that one would expect to persist in a chronic form; there are only minute ruptures in the capillaries from which the blood escapes. It is believed that the benign renal hematurias that sometimes follow tonsillitis have similar glomerular lesions; the mild nature of the lesions would explain the rapid recovery.

(*G*) *Acute Lipoid Nephrosis* (*Table I, Type 7*)

Lipoid nephrosis is usually a chronic form of renal disease, but occasionally it terminates in death several weeks after the onset. Four cases of the acute form (Nos. 76 to 79) are included in this discussion since they represent a variety of acute nephritis. The patients presented the well known clinical features, *i.e.* massive albuminuria, marked edema (except in No. 76) and absence of renal insufficiency. In 3 cases death was due to streptococcic peritonitis. The glomeruli show practically no changes except in No. 77 in which there is a rather well marked thickening of the capillary basement membranes. Although the glomeruli are histologically normal in many of these acute cases, the basic disturbance is probably injury of the glomerular capillaries. This is indicated by the increased permeability of the capillaries to plasma proteins and by the fact that the chronic cases usually develop a well marked thickening of the capillary basement membranes. Lipoid nephrosis will be discussed more fully in a subsequent publication.

Clinical Phenomena of Acute Glomerulonephritis

The 79 cases of acute glomerulonephritis may be discussed as one clinical group although the glomerular lesions vary strikingly in the different types.

Frequency: Only 42 of the 79 cases were good clinical examples of acute glomerulonephritis; 37 cases were associated with another disease and the renal lesion was considered of secondary importance. These cases were collected from a careful study of about 25,000 post-

mortems. If we consider only the cases uncomplicated by another disease, in which the glomerular lesions were of such a nature that they might have progressed to chronic glomerulonephritis, there are 42 cases. In this same series of postmortems there were 120 deaths from chronic glomerulonephritis, from which it is obvious that death from glomerulonephritis is much more apt to occur in the chronic than in the acute stages of the disease. Since acute glomerulonephritis so often terminates in recovery it is no doubt seen much oftener in clinical practice than in the autopsy room.

Age: Seegal, Seegal and Lyttle analyzed the clinical records of 381 cases of acute glomerulonephritis from several hospitals, the diagnoses being made from the hospital records. They found that about 50 per cent of the cases occurred in the first decade, about 20 per cent in the second, about 15 per cent in the third, and small percentages in subsequent decades.

Our group of uncomplicated cases is too small for statistical analysis, but over half of them were over 30 years of age.

Sex: Seegal, Seegal and Lyttle found acute nephritis about twice as frequent in males as in females, but Murphy reported 51 males and 53 females.

Duration: The duration of symptoms referable to the kidneys is shown in Table I. This is difficult to determine accurately, especially in cases complicated by another disease. In the uncomplicated cases the onset is considered the time when the patient first noticed edema or hematuria, or realized that he was ill. However, there is often a continuous illness from the onset of the initial infection (sore throat, common cold, and so on) until the appearance of edema or hematuria. There are several cases in which one may be reasonably sure that the age of the renal lesion is less than 1 week. The duration of the renal disease depends on several factors: (*a*) death may be due largely or in part to the associated disease of which the nephritis is a terminal complication; (*b*) it may be hastened by the development of bacteriemia or peritonitis during the course of the nephritis; (*c*) it may occur early in the course of nephritis from edema of the lungs or larynx; and (*d*) it may be caused by uncomplicated renal insufficiency. The cases of short duration give opportunity for study of the early stages of the glomerular lesions.

Albuminuria: The diagnosis of acute glomerulonephritis cannot be made in the absence of albuminuria. In this series protein was

found in every case in which the urine was examined, and usually in large amounts (2 to 4+). In those instances in which only a trace, 1−, is recorded the patient had another disease and the last examination of the urine was usually made several days prior to death, before the nephritis had reached its maximum intensity. In the presence of hematuria, albumin in the urine has no additional significance. The amount of albumin in samples of urine often varies greatly from time to time and does not indicate accurately the extent of the renal damage. Protein escapes from the blood through injured glomerular capillaries, but the amount of leakage is not proportional to the visible structural changes; in fact protein cannot escape from capillaries unless they are open enough to allow the free passage of blood. The most severely damaged glomeruli have closed capillaries and do not transmit any albumin. The leakage of protein is greatest through the best, *i.e.* the most open capillaries. In some cases of acute lipoid nephrosis the capillaries show no visible alterations of structure yet they allow the passage of large quantities of protein.

In general, a marked albuminuria indicates nephritis of some form, but Murphy and coworkers found no relation between the degree of albuminuria and the subsequent course of acute glomerulonephritis. Albuminuria obviously depends on glomerular and not on tubular injury.

Hematuria: Hematuria is used to denote urine that contains sufficient blood to give it a red color, or at least a reddish tinge. In 11 cases (Type 6) hematuria was the most prominent clinical evidence of renal injury, and in 11 other cases it was present but less conspicuous. Red cells were found in the centrifuged urine in most instances when they were looked for, but often they were no more numerous than in cases of passive congestion of the kidneys. Murphy found gross hematuria in 36 of 94 cases, and erythrocytes were found in the sediment at some time in every case. In some cases of hypertension with acute renal insufficiency a definite hematuria is present and erythrocytes are also found in the urine in simple passive congestion. It seems therefore that "glomerulonephritis" is a more accurate term than "hemorrhagic nephritis," which some writers employ.

The erythrocytes escape from open glomerular capillaries, not from those plugged with endothelial cells or leukocytes. In microscopic sections one sees that the erythrocytes have escaped from

capillaries that appear normal except for minute points of rupture. The fact that erythrocytes can escape from a capillary is evidence that it is not permanently damaged. In the table it appears that the cases with severe hematuria have the least glomerular damage. This corresponds with clinical experience that severe hematuria without edema, hypertension or renal insufficiency affords a good prognosis. Baehr has suggested the term "benign hemorrhagic nephritis" for this form of renal disease that heals rapidly without permanent damage to the kidneys.

Our cases of the hemorrhagic type (Nos. 65 to 75) are a peculiar form of renal disease. Although hematuria was an outstanding symptom the clinical picture indicated septicemia rather than nephritis. Renal insufficiency is produced by extensive obstruction of the tubules by blood. The renal lesion resembles that found in uremia following transfusion with incompatible blood.

Edema: The degree of edema (subcutaneous edema, ascites, hydrothorax) is roughly indicated in the table by numerals. When the amount of edema varied during the period of observation the average condition is indicated. It will be noted that edema was absent at all times in 23 cases. Eight of the 31 uncomplicated cases of the proliferative form (Type 1*a*) showed no edema. There were only 28 cases in which edema was prominent (Grades 2 to 4). Edema was inconspicuous or absent in the hemorrhagic type. In three instances (Nos. 20, 21, 25) death was due, not to renal insufficiency, but to edema of the lungs and larynx. Several writers have reported cases of acute nephritis in which death was due to edema of the lungs. Murphy observed edema in 62 of 94 patients but it was never a severe complication. The presence of edema is not necessary to establish the diagnosis of any type of acute nephritis except lipoid nephrosis.

The factors that influence the development and the intensity of edema in acute glomerulonephritis are not well understood. The plasma proteins were not determined in our cases. The depletion of plasma proteins may be a factor of importance in some instances, but according to the majority of investigators they are at a normal level or only slightly reduced at the height of the edema. In 5 of our cases (hypertension and bacterial endocarditis) the severe edema was probably due chiefly to cardiac decompensation.

Some writers have described acute dilatation of the heart with

cardiac decompensation in acute glomerulonephritis (Franke, Levy). This has been noted especially in patients with marked hypertension. The cardiac dilatation was demonstrated in roentgenograms. In 3 of 27 fatal cases Murphy attributed death to heart failure, and in 15 of his cases myocardial insufficiency of varying degrees was noted. Koch reported that in 3 of 7 fatal cases of subacute glomerulonephritis death was caused by cardiac failure.

In 24 of our 31 uncomplicated cases of acute proliferative glomerulonephritis sections of liver were available for microscopic examination and 13 of these showed chronic passive congestion of some degree, as shown in Table I. Passive congestion was absent in 11, slight in 9, moderate in 2, and severe in 2 instances. There is, however, no close correlation between edema and chronic passive congestion of the liver; among 17 patients with edema only 9 had passive congestion and 2 of the 8 without passive congestion had severe edema. An increase of venous blood pressure is frequently demonstrable in acute glomerulonephritis (George Fahr).* It appears that cardiac failure is partly responsible for edema but it does not seem to afford a complete explanation. Edema fluids in acute glomerulonephritis are usually said to have a high protein content suggesting an inflammatory origin, but Fahr and Kerkof found a low protein content when they were very careful in obtaining pure edema fluid for examination. Certainly the protein content of the edema fluid in acute glomerulonephritis is much lower than that of known inflammatory exudates.

Blood Pressure: The blood pressure was recorded in 47 of the 79 cases. The highest recorded blood pressure was tabulated unless it was inconsistent with other readings. The systolic pressure was below 140 mm. Hg. in 16 instances, 140 to 150 in 7, 150 to 170 in 9, 170 to 200 in 9, and over 200 in 6. Six of the cases with a blood pressure above 190 mm. Hg. were known to be instances of primary hypertension in which acute glomerulonephritis was a terminal complication. The low blood pressure in some instances was probably due to its having been recorded only a short time before death; it is known that the blood pressure often falls in the terminal stages of any disease. The patient may pass into uremia whether the blood pressure is high, low or normal. Murphy and coworkers found hypertension in 74 of 94 patients; they think that hypertension has no re-

* Personal communication.

lation to the outcome of the disease unless it persists after other symptoms have subsided, in which case it indicates a tendency to chronicity. It is generally agreed that hypertension may not be present at all times in acute glomerulonephritis and that it may be absent entirely. In mild cases it may be present only during the first few days. It usually subsides long before albumin disappears from the urine. No case can be regarded as healed if hypertension is still present. Persistent hypertension beyond the acute stage indicates the development of chronic nephritis.

Hypertension is probably due to a diminished flow of blood through the kidneys. Bell and Pederson produced hypertension experimentally by obstructing one renal vein, and Goldblatt and coworkers produced chronic hypertension by narrowing both renal arteries. The experimental evidence supports the view that anemia of the kidney is the primary cause of the rise of blood pressure. It has been shown that hypertension occurs in Goldblatt's experiment with denervated kidneys (Page, Goldblatt *et al.*) so that it does not depend on reflexes from the anemic kidney. In acute glomerulonephritis there is a reduced flow of blood through the kidneys brought about by the widespread capillary obstruction, and it appears probable that either the anemia of the kidney or the increased resistance in the renal circulation is in some way responsible for the hypertension. The absence of hypertension in some severe cases of acute glomerulonephritis is presumably due to a failure of the heart or vasomotor system to respond to the stimulus from the diseased kidney. Volhard believes that arteriolar spasm in the kidneys precedes and causes the structural changes in the glomeruli, but the glomerular lesions develop in the absence of hypertension and they are not the type of lesion that develops elsewhere from ischemia.

Renal Insufficiency: In fatal cases renal function decreases rapidly during the last few days and the highest level of blood urea is observed on the last day of life. The blood urea nitrogen may be found normal or only moderately elevated 1 week before death but at a uremic level on the last day. The number of days or weeks before death when the functional test was made is indicated in the table, and it will be noted that a high blood urea nitrogen was nearly always found in the cases that were tested shortly before death. It is apparent that renal failure is usually the chief cause of death. However, the patient may develop edema of the lungs or larynx

(No. 25), or a terminal septicemia (Nos. 52 and 75), and die before the blood urea is much elevated. In the cases of acute lipoid nephrosis (Nos. 76 to 79), death was due to peritonitis in 3 instances. The associated disease was the main cause of death in several cases.

In 6 of the 7 cases with severe hematuria (Type 6) in which the blood urea nitrogen was determined it was found to be markedly elevated. These patients were suffering from bacteremia rather than from renal disease and the renal insufficiency was brought about by extensive obstruction of the tubules with blood.

Murphy found nitrogen retention in 67 of 94 cases. A patient may recover even after blood urea has reached high values. Jauréguy and Ayala reported the recovery of a patient whose blood urea was 115 mg. per cent. Functional tests give the functional capacity of the kidneys at the time they are made but they are not a safe criterion for prognosis in acute nephritis since the renal function may rapidly improve or deteriorate.

The most reasonable explanation of impaired renal function in acute glomerulonephritis is based on the structural changes in the glomeruli. In severe cases a large proportion of the glomerular capillaries are closed more or less completely by endothelial cells and leukocytes, or by external pressure from epithelial crescents. Since the closed capillaries contain no blood they cannot take part in the formation of glomerular filtrate, and if nearly all the capillaries are closed anuria must result. However, a few open capillaries are usually found in most of the damaged glomeruli and in some nearly all the capillaries are open. The glomerular filtrate is formed from the open capillaries and presumably filtration is taking place through all open capillaries. The total glomerular filtrate is decreased in amount in severe cases and it is distributed through more tubules than under normal conditions in which only about one-third of the nephrons are active at any one time. The decrease of glomerular filtrate tends to cause retention of metabolites. The distribution of the filtrate through a larger number of tubules where it is exposed to greater reabsorption tends to produce a concentrated urine. With greater tubular reabsorption it is probable that more urea passes back into the blood. A similar explanation of abnormal renal function has been offered by Dunn and by Fremont-Smith and his associates.

Protein must escape through open glomerular capillaries and

heavy proteinuria does not necessarily indicate irreparable injury. The amount of protein in the urine decreases in contracted kidneys since there is a great reduction in the number of open capillaries.

The erythrocytes escape from minute ruptures of open capillaries and hematuria would therefore have no serious significance except for the obstructive lesions that may be associated with it.

Another factor that should be given consideration in determining the anatomical basis of disturbed renal function is the swelling of the kidneys. The kidneys are nearly always much larger than normal and their capsules are tense. The swelling is not due to an increased amount of blood, since the tissue is pale and microscopically the blood content seems less than normal. However, there is some enlargement of the tubules from swelling of their cells or from dilatation of their lumens which tends to compress the intertubular capillaries. Occasionally there is some interstitial edema which also tends to compress capillaries and reduce the rate of blood flow.

Relation to Infection: Our observations agree with those of nearly all observers that there is usually a history of infection preceding acute glomerulonephritis. By far the most common antecedent infections are those of the upper respiratory tract, *i.e.* sore throat, common cold, bronchitis, scarlet fever, and so on. A large variety of infectious processes appear occasionally immediately preceding the nephritis, *i.e.* smallpox, chickenpox, infected wound, impetigo contagiosa, and so on. In most instances the antecedent infection produces a lesion of mucous membrane or skin which allows the entrance of bacteria, especially streptococci into the tissues. Proliferative glomerulonephritis is usually caused by streptococci, less frequently by pneumococci or other organisms. The difficulty in establishing the exact etiological agent is due to the uniform absence of bacteria from the glomerular lesion. It appears that the diffuse glomerular lesions are produced by a soluble toxic substance and that if the bacterial bodies lodge in the glomeruli an embolic type of lesion results.

The interval between scarlet fever and the onset of nephritis is usually 2 to 3 weeks and this fact has suggested to several investigators that the glomerular lesion does not develop until the individual has become hypersensitive to the bacterial protein. Fahr and Masugi have sought to establish this principle by demonstrating that a severe glomerulonephritis results from injection of foreign

serum into the renal artery of an animal previously sensitized to the corresponding serum. A typical glomerulonephritis may indeed be produced in this way but it seems more comparable to the Arthus phenomenon than to glomerulonephritis.

In many instances the interval between the initial infection and the onset of symptoms of nephritis is too short for sensitization to have developed.

II. Vascular Lesions

Acute renal insufficiency may be produced by thrombosis of the renal arteries or by widespread thrombosis of small arteries with formation of multiple infarcts.

(*A*) *Thrombosis of Renal Artery*

The patient (35–1943), a female, 71 years of age, had had a right nephrectomy for calculus 30 years previously. She developed extreme oliguria and died of uremia. The left renal artery was almost occluded by an old thrombus and there were numerous small infarcts throughout the kidney.

(*B*) *Widespread Thrombosis of Small Arteries*

Male (35–730), 9 years old. During the course of an empyema following pneumonia the patient developed anuria and died of uremia. The systolic blood pressure was 120 mm. Hg. The blood urea nitrogen was 182 mg. per cent on the day before death. There was no edema. The kidneys weighed 350 gm. and were filled with small infarcts caused by thrombosis of small arteries. The greater part of the cortex of both kidneys was necrotic. Cortical necrosis of the kidneys has been reported frequently in eclampsia and a similar lesion has been described in males associated with infectious processes.

(*C*) *Polyarteritis Nodosa*

Male (32–588), 64 years old. The duration of the illness was about 3 weeks. There was no albuminuria or edema. The urinary output was not noted. The renal disease was not recognized clinically but the extensive infarction of the kidneys found at postmortem is sufficient to establish the diagnosis of renal insufficiency. The kidneys weighed 400 gm. The infarcts were related to lesions of the

small arteries. It is well known that patients suffering from polyarteritis nodosa may develop uremia from extensive lesions of the small renal arteries.

III. Interstitial Lesions

(*A*) *Acute Interstitial Nephritis*

A male (25–1059), 23 years of age, developed sore throat, fever and general malaise on December 12th. On December 19th he was admitted to the hospital with a typical scarlet fever rash and sore throat. At this time the urine was negative. He improved for a few days and then became very toxic on December 24th. The blood pressure was 80/42. His condition gradually became worse and death occurred on December 30th. The urine showed only a trace of albumin until December 29th, when there was a heavy albuminuria with many casts and leukocytes. There is no record as to the diuresis. On December 30th the blood urea nitrogen was 174.3 mg. per cent and creatinine 10.6 mg. per cent.

The kidneys were enormously enlarged, the right weighing 520 gm., the left 570 gm. Microscopically there was a massive infiltration of the interstitial tissues with mononuclear and polymorphonuclear leukocytes. The renal insufficiency was probably due to compression of the blood vessels and tubules throughout the kidneys by the massive interstitial exudate.

(*B*) *Acute Lymphatic Leukemia*

The patient (33–1069), a female, 40 years of age, was well until June 1, 1933, when she first noticed purpura and discoloration following slight bruises. On June 12th there was a severe hemorrhage following extraction of a tooth. She was admitted to the hospital June 17th, complaining of weakness and petechial hemorrhages over the greater part of the body. The blood picture was that of lymphatic leukemia. The urine showed heavy albuminuria and many erythrocytes. There was no edema. June 17th the blood urea nitrogen was 105.4 mg. per cent and the creatinine 7.5 mg. per cent. Death occurred June 20th, 1933. The kidneys weighed 370 gm. and showed widespread leukemic infiltration of the cortices. The effect on the kidney is similar to that of acute interstitial nephritis.

IV. Tubular Diseases of the Kidneys

(*A*) *Pure Tubular Degeneration*

(*a*) *Mercuric Chloride Nephritis:* There are 7 cases of mercuric chloride poisoning in our records and in 6 of these death was due to renal insufficiency.

1. A male, 51 years of age, who was suffering from carcinoma of the colon, took an unknown amount of mercuric chloride and died 30 minutes later. At postmortem the kidneys showed no gross or microscopic alterations. The kidneys are presumably injured but some hours must elapse before any structural changes can be detected.

2. A male, 81 years of age, was admitted to the hospital 2 days before his death. He was uncertain as to when he took the poison. No urine was voided but 50 cc. was removed by catheter. This contained only a trace of albumin. There was no edema. The blood urea was 218 mg. per cent. At postmortem no colitis was found. The kidneys weighed 334 gm. Many cells in the convoluted tubules are necrotic and desquamated, but nearly all the tubules are lined with fairly thick, deeply stained cells. The lumens are not dilated.

3. A female, 45 years of age, died 7 days after taking an unknown amount of mercuric chloride. Very little urine was passed during this period. There was no edema. At postmortem ulcerative colitis and ileitis was found. The kidneys weighed 350 gm. The convoluted tubules are not distended but their lumens are filled with necrotic and desquamated cells. Numerous partly formed epithelial casts are noted. There is no evidence of tubular regeneration; there are no dark flattened cells lining the tubules.

4. A female, 29 years of age, died 10 days after inserting a 5 gr. tablet of mercuric chloride into her vagina. She complained of a severe burning pain, chills, fever and headache. The blood pressure was 110/70 on the 2nd day, and 98/55 on the 3rd day. Ulceration of the gums developed on the 7th day. No urine was voided except a small quantity on the 6th and 7th days, which contained some albumin and casts. On the 7th day there was edema of the right optic disc and a slight subcutaneous edema. At postmortem gangrenous cystitis, ulcerative colitis and acute pericarditis were found. The kidneys were enormously enlarged, weighing 825 gm. The con-

voluted tubules are all enormously dilated and lined by very thin epithelial cells that contain no granules. In some tubules the lining cells are completely absent, but no necrotic cells are to be seen (Fig. 7). The few casts that are present seem entirely inadequate to explain the tubular distention.

5. A female, 24 years old, died 11 days after taking 10 gr. of mercuric chloride. Some of the poison was removed by vomiting and gastric lavage. There was no record of the diuresis. The urine contained a small amount of albumin. At postmortem a non-ulcerative colitis was found. The kidneys weighed 370 gm. There are a few necrotic tubules. There is not much tubular dilatation and the tubules are lined with fairly thick dark cells.

6. A female, 14 years of age, took 22.5 gr. of mercuric chloride. She vomited shortly afterwards. Oliguria developed on the 2nd day and persisted in a severe form until the 10th day, but there was some increase of the urinary output during the last 3 days. The gums became sore on the 3rd day. On the 13th day the blood pressure was 104/50. The blood urea nitrogen was 74.6 mg. per cent on the 4th day, and 124 mg. per cent on the 10th day. There was no edema. Death occurred on the 13th day. The kidneys weighed 370 gm. There is a heavy lymphocytic infiltration in the medullae. The tubules are markedly dilated and lined by thin dark epithelium. No necrotic cells are visible and there are only a few casts.

7. A female, 26 years of age, died 16 days after taking 25 gr. of corrosive sublimate. She complained of severe abdominal pain and became semicomatose on the 2nd day. A severe stomatitis developed. The systolic blood pressure was 126 mm. Hg., on the 2nd day, 164 mm. Hg. on the 10th day, and 128 mm. Hg. on the 16th day. The blood urea nitrogen was 20.5 mg. per cent on the 2nd day, 140 mg. per cent on the 9th day, and 162 mg. per cent on the 15th day. There was a heavy albuminuria at first but later only a trace was present. There was severe oliguria at first but during the latter part of the illness the urinary output was about 1000 cc. daily. The specific gravity of the urine was never above 1014. There was no edema. The hemoglobin decreased from 90 to 65 per cent.

At postmortem a few small areas of ulcerative colitis were found. The kidneys weighed 370 gm. The convoluted tubules are all dilated and lined by low dark epithelial cells (Fig. 8). A few necrotic

tubules are noted. There are numerous casts in the collecting tubules. There is a definite interstitial edema and an occasional glomerular abscess is noted.

The most prominent symptom in corrosive sublimate poisoning is oliguria which usually progresses to anuria. As a result of the decreased diuresis there is a progressive increase of urea and other blood metabolites and death results from uremia. Occasionally, even in cases that terminate in uremia, there is an increased diuresis after several days (Case No. 7) and the urinary output may approach the normal. In such instances recovery may take place (Mach and Oppikofer) or the blood urea may continue to increase as in our Case 6. It will be noted in our case that the kidneys were unable to concentrate the urine above 1014. With such a loss of concentrating power a diuresis of 1000 cc. daily is insufficient to prevent the increased accumulation of blood metabolites.

There is usually a large amount of albumin in the urine during the first day or two, but later there is very little. This indicates that there is a primary glomerular injury which soon subsides.

Edema is usually absent entirely or very slight in amount, but when large quantities of fluid are given it may become very marked (Sollmann and Schreiber).

The blood pressure is sometimes moderately increased during the stage of severe oliguria. The blood urea increases steadily in the cases that terminate fatally. In the case reported by Mach and Oppikofer the blood urea reached 644 mg. per cent on the 11th day, but the patient recovered. The high level of the blood metabolites is due in large measure to renal failure, but the depletion of the body fluids by vomiting and diarrhea is a contributory factor. Roth found a striking decrease of blood chloride in his patient and attributed the uremia to hypochloremia.

In persons who die within a few hours after taking the poison no renal changes are demonstrable. In those who die after several days there is usually found a marked necrosis and desquamation of the cells of the convoluted tubules. Not all the cells in the tubules become necrotic; many survive. Often it appears that the inner half or two-thirds of the cell becomes necrotic and desquamates while the basal portion containing the nucleus persists. Regeneration probably takes place from the basal portion of the cell.

In those who die 10 or more days after ingestion of the poison the

kidney presents quite a different microscopic appearance. Very little necrosis is now seen. The tubules are lined with cells that appear much darker than the normal cells in hematoxylin-eosin preparations. The mitochondrial granules are not to be seen. The cells are usually flattened or cubical in shape (Fig. 8) and the lumens of the tubules are usually widened. The dark cells are interpreted as new epithelium formed by regeneration. The decreased height of the cells is partly responsible for the wide lumen, but there are numerous casts in the collecting tubules which may obstruct the outflow of urine. There is often some interstitial edema.

The glomeruli show no structural changes. Evidently there is a moderate initial glomerular injury since there is heavy albuminuria during the first day or two, but the disappearance of albumin later indicates only temporary injury. In frogs poisoned with mercuric chloride Richards found the glomerular circulation normal although no urine reached the renal pelvis. By injections with Janus green Moore and Hellman concluded that there is no decrease in the number of open glomeruli in the anuric stage of mercuric chloride nephritis.

Richards demonstrated in the frog's kidney that in mercuric chloride poisoning the glomerular filtrate is formed in normal amount and composition. It evidently diffuses back into the interstitial tissue through the denuded tubular wall or through cells that have no power of selective retention. Phenol red diffuses back into the tissue when injected into the tubule. One must suppose that the regenerated cells seen in the human renal tubules in the later stages of the disease have not yet attained a normal functional capacity.

It is clear that mercuric chloride nephritis is a purely tubular disease and that the characteristic disturbance in tubular disease is oliguria or anuria and not albuminuria or edema. The conception of lipoid nephrosis as tubular disease finds no support in the study of the mercuric chloride kidney.

(*b*) *Other Types of Pure Tubular Degeneration*

8. 33–1730. A male, 56 years of age, had had intermittent nausea and diarrhea for 4 years prior to admission. He died 11 hours after he entered the hospital. The blood pressure was 82/50, and no urine could be obtained by catheter. The blood urea nitrogen was 116 mg. per cent, and the van Slyke 26 volumes per cent. He was consider-

ably emaciated. At postmortem 300 cc. of urine was obtained from the bladder and this showed a specific gravity of 1010, albumin + and a few erythrocytes. There was a terminal bronchopneumonia. The kidneys weighed respectively 160 gm. and 180 gm. Microscopically there is found a severe hydropic degeneration of all the convoluted tubules. There is no glomerulitis and the tubular lesions are severe and amply sufficient to explain the uremia. The duration of the renal disease cannot be determined from the meager clinical history.

9. 35–483. An obese white female, 24 years of age, was admitted to the hospital on March 17th. For 10 days prior to admission she had been confined to bed with chills and fever. She had vomited occasionally and had a marked oliguria. On admission she was semicomatose; the blood pressure was 130/80, and the temperature 99.2° F. The urine was always scanty in amount and contained a large amount of albumin. The hemoglobin was 87 per cent, and there were 16,500 leukocytes per cmm. On March 19th the blood urea nitrogen was 109 mg. per cent, and creatinine 1.8 mg. per cent. On March 22nd the blood urea nitrogen was 123 mg. per cent, and the plasma chlorides 472 mg. per cent. Death occurred on March 23rd.

At postmortem there was found a purulent pericarditis and a moderate bronchopneumonia, both of which appeared to be terminal secondary infections. The kidneys weighed 290 gm. each and were very pale. Microscopically most of the convoluted tubules show extreme hydropic degeneration (Fig. 9) and the remainder are greatly dilated and lined by a thin epithelial layer. This is a pure degenerative tubular lesion. There is no glomerulitis. There is no resemblance to the mercuric chloride kidney, and careful questioning of the relatives of the patient failed to bring out anything suggestive of any type of chemical poisoning.

(*B*) *Obstructive Tubular Disease from Blood Transfusion*

10. 31–1823. A male, 51 years of age, was admitted to the hospital October 7th, with an inoperable carcinoma of the stomach. His hemoglobin was 34 per cent and his red cell count 1,740,000. On October 19th a blood transfusion was begun. After 100 cc. had been given the transfusion was discontinued because the patient went into shock. It was later determined that the donor belonged to Group II

and not to Group IV as did the recipient. The patient recovered from shock but developed an oliguria which persisted. In spite of a large fluid intake the urinary output was 200 cc. on October 20th, no urine was obtained on October 23rd, 400 cc. on October 25th, 575 cc. on October 29th, but very little urine after this date. On October 29th, no phenolsulphonephthalein was excreted in 2 hours. The non-protein nitrogen was 151 mg. per cent. Death occurred November 2nd.

The kidneys weighed 250 gm. each. On section the surfaces were pale. Microscopically a majority of the collecting tubules are plugged with hemoglobin casts and there is a moderate distention of all the convoluted tubules. There are no other changes.

11. 34–1183. A female, 29 years of age, was admitted to the hospital June 12th. She had induced an abortion about 2 weeks previously and now had puerperal septicemia. The hemoglobin was 40 per cent and the leukocyte count was 9000. There was continuous fever. The blood pressure was 120/80. The urine showed only a faint trace of albumin. On June 19th a blood transfusion was given, but before the transfusion was completed the patient developed a severe shock. She had been given 360 cc. of citrated blood from her husband. The preliminary tests indicated that both donor and recipient belonged to Group I. She recovered from the shock but during the next 24 hours passed only 50 cc. of urine The urine showed a specific gravity of 1035, a 4 plus albumin and a large amount of hemoglobin. Although hemoglobin soon disappeared from the urine a marked oliguria persisted. In spite of a large fluid intake and the use of spinal anesthesia the output of urine varied from one-fourth to one-tenth of the fluid intake. A heavy albuminuria persisted after disappearance of the hemoglobin, and there were many erythrocytes in the urine. The hemoglobin remained around 30 per cent and the erythrocyte count about 2,000,000. On June 20th the icterus index was 30 and a quantitative determination of bilirubin gave 19 parts per million. The blood urea nitrogen was 52 mg. per cent on June 20th, 90 on June 29th, 115 on July 3rd, and 144 on July 5th. During this time blood creatinine rose from 3.5 mg. per cent to 9.9. On July 9th dyspnea developed which was found to be due to edema of the lungs. The patient did not become drowsy but remained awake and alert until death on July 9th.

At postmortem there was found marked edema of the lungs and

500 cc. of clear fluid in the peritoneal cavity. The kidneys were enlarged, weighing 215 gm. and 240 gm. respectively. The cortices were gray in color. Microscopic examination shows a rather marked dilatation of all the renal tubules and in some parts of the cortex there is interstitial edema (Fig. 10). There is no glomerulitis and none of the tubules is necrotic. A large number of the collecting tubules and loops of Henle are obstructed by hemoglobin casts (Fig. 11).

12. 36–224. A female, 27 years of age, underwent a Cesarean section because of placenta previa. Following the operation five blood transfusions were given, each about 600 cc. The first two transfusions were given 12 hours and 18 hours respectively after the operation; the other three were given at 24 hour intervals. There are no details available as to the reaction after the transfusions. The patient developed a severe oliguria, about 60 cc. of urine daily, and died 1 week after the operation. The blood urea nitrogen rose to 120 mg. per cent. Only sections of the kidneys were available for study. The microscopic appearances are the same as in the two previous transfusion kidneys; there is a marked dilatation of all the tubules with some interstitial edema, and the collecting tubules are plugged with hemoglobin casts.

It has been known for many years that hemoglobinuria may follow blood transfusions. After the recognition of the blood groups it was determined that hemolysis and hemoglobinuria rarely occur unless the patient is transfused with blood from a donor of a different blood group. Ponfick (1875) transfused various animals with blood from other species and frequently obtained hemoglobinuria, anuria and death of the animals. He observed casts of hemoglobin in the tubules. Lemke (1925) reported a patient who died 6 days after transfusion; there were oliguria, hemoglobinuria and clinical signs of uremia. He noted some injury of the tubular epithelium and hemoglobin masses in the tubules.

The most frequent cause of death in blackwater fever is anuria and uremia, and this has been attributed by several investigators to plugging of the renal tubules with hemoglobin casts. The kidneys in such cases of blackwater fever resemble the transfusion kidneys rather closely. Yorke and Nauss (1911) were able to produce suppression of urine and uremia in rabbits by intravenous injections of hemoglobin. The renal tubules, especially the collecting tubules, were plugged with casts largely composed of hemoglobin. The kid-

neys of the experimental animals correspond entirely with those from blackwater fever and those resulting from transfusion of incompatible blood. It is important to note that suppression of urine in the rabbits was obtained only when they were on a dry diet; it did not result when they had free access to water. The authors believed that the dry diet caused concentration of the urine which resulted in precipitation of hemoglobin. Baker and Dodds (1925) studied the kidneys of two persons who died following transfusion with incompatible blood. They attributed the uremia to obstruction of the tubules by casts of hemoglobin. They confirmed the experimental observations of Yorke and Nauss, but noted that the urine from the rabbits with uremia was of a dark brown color and highly acid, while the rabbits in which no urinary suppression developed had a reddish alkaline urine. By feeding rabbits on a diet of oats and bread, without any green food, they kept the urine permanently acid although the animals were allowed unlimited water to drink. Injections of hemoglobin into rabbits with an acid urine caused urinary suppression. Hemoglobin is precipitated from acid urine.

Lindau (1928) gave a good discussion of the clinical symptoms and reported the postmortem findings in 3 cases. He found moderate injury of the convoluted tubules in 2 cases and severe degeneration in 1. The tubules were not dilated. He noted casts in the tubules but considered degeneration and necrosis of the tubular epithelium the chief cause of the anuria.

Halter (1930) described the kidneys of a patient who died of uremia on the 10th day after a blood transfusion. He noted that the tubules were dilated and that many of them, especially those in the medulla, were plugged with hemoglobin and other forms of casts. He attributed uremia to mechanical obstruction of the tubules. Irsigler, 1932, agreed with Halter that mechanical obstruction of the tubules by casts is the cause of tubular dilatation and uremia. He noted also a little lymphocytic infiltration of the interstitial tissue.

Bordley (1931) reported three severe transfusion reactions, one of which resulted fatally. The kidneys in this instance weighed together 589 gm. The tubules were dilated and there was edema and lymphocytic infiltration of the interstitial tissue. There were many necrotic epithelial cells. Casts were evidently not considered an important cause of the renal insufficiency.

Hesse and Filatov (1932, 1933) proposed the theory that uremia is due to spasm of the renal arteries. They performed experiments on dogs which showed a decrease of kidney volume following transfusion unless the kidneys were previously denervated. They recommended, as therapeutic procedures in uremia following transfusion, denervation of the kidneys and transfusion with compatible blood to release the vascular spasm.

It is to be noted in connection with this hypothesis that spinal anesthesia in one of our cases (No. 11) had no influence on the oliguria. Johnson and Conway had previously reported that spinal anesthesia was without effect.

Johnson and Conway (1933) described the kidneys of a patient who died of uremia on the 18th day after a transfusion. Dr. F. B. Mallory found areas of focal necrosis in the liver and adrenals; the kidneys showed severe damage of the tubular epithelium with evidences of regeneration.

DeGowin and Baldridge (1934) described and illustrated the changes in the kidneys in a patient who died on the 10th day following transfusion. The collecting tubules of the medulla were filled with hemoglobin casts; the convoluted tubules were moderately dilated and showed a low cuboidal epithelium resembling the late stage of mercuric chloride poisoning. There was a notable interstitial edema in the cortex.

Terplan and Javert (1936) described the kidneys from a case of hemoglobinuria with fatal uremia following excessive administration of quinine in early pregnancy. The collecting tubules were obstructed by hemoglobin casts as in the transfusion kidneys.

In my 11 cases of hemorrhagic nephritis the tubules were obstructed for the most part by compact masses of erythrocytes but in 1 case many of the casts were composed largely of hemoglobin.

It appears that suppression of urine and uremia following transfusion with incompatible blood is due in large measure at least to mechanical obstruction of the tubules by casts of hemoglobin. The explanation offered by Baker and Dodds that the acidity of the urine is the determining factor in causing the precipitation of hemoglobin in the renal tubules seems well established by their experiments. In the light of their work alkalinization of the urine preliminary to transfusion might be worth while in case there is any uncertainty as to the compatibility of the blood to be used.

V. Extrarenal Uremia

Extrarenal uremia refers to uremia that occurs as a result of extrarenal influences when the kidneys are structurally normal. This form of uremia is not at all uncommon and will be found frequently if the blood urea nitrogen is determined during the last days of life or immediately after death. Frequently clinical evidences of uremia are present. In many instances of extrarenal uremia the kidneys are found to be entirely normal or only slightly altered; in other cases there are renal changes such as enlargement and dilated tubules but the structural changes are inadequate to explain renal insufficiency.

(*A*) *With Some Tubular Injury*

13. 33–2051. A female, 39 years of age, underwent a vaginal hysterectomy on November 21st. Her temperature rose on the 8th postoperative day and continued to be elevated thereafter. On November 28th the urine contained a large amount of albumin and many pus cells and erythrocytes. Several subsequent examinations of the urine gave similar findings. There was no record of the quantity of urine. On December 12th only a trace of phenolsulphonephthalein was excreted in 2 hours. Death occurred on December 13th. Blood taken 4 hours after death showed urea nitrogen 113 mg. per cent, and creatinine 4 mg. per cent.

At postmortem a pelvic abscess was found and there was a moderate amount of bronchopneumonia. The kidneys weighed 110 gm. and 210 gm. respectively. Microscopically there is a widespread severe hyaline granular degeneration of the convoluted tubules but no necrosis. There is no glomerulitis. In the medulla there are some casts and some foci of lymphocytes. In this case there is evidence that tubular injury is partly responsible for the renal insufficiency.

14. 36–235. A male, 73 years of age, sustained a severe injury of his right leg on January 20th, which was followed by severe infection of the entire leg below the knee. He was admitted to the hospital on January 30th and the infection was treated by hot packs and incisions. His temperature gradually rose to 107.8° F. and he died on February 4th with evidence of septicemia. The urine showed a little albumin and many casts (no note on the diuresis). The hemoglobin was 73 per cent and the leukocyte count 31,500 (93 per cent poly-

morphonuclears). The blood urea nitrogen was 169 mg. per cent on February 2nd.

Postmortem examination revealed severe cellulitis of the leg, a severe portal cirrhosis of the liver and terminal bronchopneumonia. The kidneys weighed 170 gm. and 190 gm. each and their cortices were cloudy. Microscopically the cells of the convoluted tubules are all swollen and vacuolated but there is no necrosis and no dilatation (Fig. 12). There is no glomerulitis. The tubular injury is not sufficient to explain the uremia and we must therefore suppose that some extrarenal factor is partly responsible.

15. 36–468. A male, 34 years of age, had taken an unknown amount of phenobarbital. He fell and sustained a wound of the scalp in the occipital region. He was admitted to the hospital immediately afterwards in coma. It was not known whether the coma was caused by the fall or by the drug, but the postmortem revealed no fracture of the skull or bruising of the brain. On admission January 31st he was in extreme shock; the blood pressure was 60/0. The patient slowly recovered from shock but remained in a stuporous condition until his death on February 9th. The urinary output was 900 cc. on January 31st, but thereafter the daily diuresis varied from 60 cc. to 190 cc. The total daily fluid intake varied from 2500 cc. to 3890 cc. He vomited occasionally. On February 5th a generalized anasarca developed. The blood urea nitrogen was 38.9 mg. on February 2nd and 63 mg. per cent on February 4th; creatinine on corresponding dates was 3.8 mg. per cent and 5 mg. per cent. The blood pressure on February 5th was 170/86. The right kidney was decapsulated on February 7th, but there was no improvement in the diuresis. Death occurred on February 9th in clinical uremia. The urine showed a large amount of albumin after February 2nd.

The kidneys weighed 210 gm. and 205 gm. each. Microscopically about half of the convoluted tubules are markedly dilated and lined by thin flattened epithelium, but the others appear fairly normal. There are many casts in the collecting tubules which are probably sufficient to explain the dilated cortical tubules. There is no glomerulitis and there is no necrosis. The uremia is largely due to extrarenal factors but tubular obstruction is a contributory factor.

16. 34–1449. A female, 35 years of age, became ill on August 8th with nausea, severe vomiting and epigastric pain. On August 13th jaundice appeared. On admission, August 15th, the temperature

was normal and the blood pressure 140/86. There was a slight jaundice. The specific gravity of the urine was 1026 and there was a large amount of albumin. There was a persistent oliguria (about 400 cc. daily) and subsequent urine examinations showed a specific gravity from 1011 to 1015, and moderate to severe albuminuria. The blood urea nitrogen on August 22nd was 175 mg. per cent and creatinine 8.8 mg. per cent. Death occurred on August 23rd.

At postmortem there was a slight jaundice but no edema. The liver showed areas of necrosis and moderate fatty metamorphosis. There were multiple small abscesses in the pancreas. The kidneys were greatly enlarged, weighing 290 gm. and 300 gm. each. Microscopically the cortical tubules are all moderately dilated and most of the collecting tubules and loops of Henle are obstructed by casts. There is some lymphocytic infiltration in the medullae. There is no glomerulitis. The obstruction of the tubules by casts is a definite contributory cause of the uremia.

17. 34–919. A male, 51 years of age, was admitted to the hospital on May 19th. For several years he had drunk large quantities of alcoholic liquors, often as much as one quart daily. For 5 days preceding admission he had been unable to eat, had been vomiting steadily and had passed no urine for the last 3 days. The blood pressure was 150/84. The sclerae showed a slight jaundice. The vomiting persisted. He passed no urine and only 30 cc. was obtained by catheter during the 3 days he was hospitalized. He was given large amounts of fluid by hypodermoclysis and intravenously. The urine showed a trace of albumin. The icteric index was 156 on May 20th. On May 21st the blood urea nitrogen was 140 mg. per cent and creatinine 10 mg. per cent. The hemoglobin was 84 per cent and the red cell count 4,820,000. The temperature remained normal. The patient died in coma on May 22nd.

At postmortem the body was obese, the liver weighed 2065 gm. and was very fatty, and there was terminal bronchopneumonia. The kidneys weighed 277 gm. and 288 gm. each; their cortices were thick and cloudy. Microscopically there are no renal changes except some swelling and vacuolization of the cells of the convoluted tubules. This is evidently chiefly an extrarenal uremia from loss of fluid but the marked swelling of the kidneys indicates some renal injury.

18. 34–1059. A male, 36 years of age, was admitted to the hospital on June 5th. His illness began 10 days previously with vomiting.

He could retain no solid food and only a small amount of liquid. This condition continued until his death on June 17th. He was given fluids subcutaneously and intravenously but continued to vomit. The specific gravity of the urine ranged from 1010 to 1018, and the amount of albumin varied from 1 to 4 plus. There was no record of the amount of diuresis but he did not have anuria. There was no excretion of phenolsulphonephthalein in 2 hours. His blood pressure was continuously about 180/110. The hemoglobin was 76 per cent, and the leukocyte count rose from 9500 to 19,000. On June 13th the blood urea nitrogen was 189 mg. per cent and creatinine 8.3 mg. per cent. Death occurred on June 17th.

At postmortem the only notable changes were in the kidneys which weighed 250 gm. each. Microscopically there is no glomerulitis, the tubules are all moderately dilated but there are only a few casts and there is no necrosis. The tubule cells are not deeply stained, as they are in late bichloride poisoning.

This appears to be largely extrarenal uremia from loss of fluids but the kidneys are not normal.

19. 34–1901. A white female, 30 years of age, was admitted to the hospital on November 1st in diabetic coma. She was known to have had diabetes for several months but had received no treatment. Coma developed on the afternoon of October 31st. She had been in coma about 10 hours when admitted. The blood pressure was 70/40 and the breathing was of Kussmaul type. The urine showed specific gravity 1020, albumin 2 plus, sugar 4 plus, acetone and diacetic acid. The blood sugar was 625 mg. per cent and the van Slyke 8 volumes per cent. Under treatment the blood pressure soon rose to 110/60, blood and urine sugar were decreased, and she slowly regained consciousness. There was oliguria at first but this improved somewhat by the 3rd day. The blood urea nitrogen was 41 mg. per cent on November 1st, 56 on November 2nd, and 68 on November 3rd. On November 2nd the phenolsulphonephthalein output in 2 hours was only 2 per cent. Death occurred on November 5th.

The kidneys were enormously enlarged, weighing 280 gm. and 320 gm. respectively. Microscopically the only notable change is a uniform, rather marked tubular dilatation (Fig. 13). There are only a few casts. The interpretation is extrarenal uremia with contributory renal injury.

20. 30–1937. A male, 18 years of age, was admitted to the hospital

December 21st. He had had rather severe diabetes for 2½ years, and had had several attacks of coma. He had been under insulin treatment but had been careless in his self management. On the day of admission he had become weak, sleepy and tired, and had begun to vomit. He developed dyspnea and orthopnea. The urine showed albumin 4 plus, many casts, sugar 4 plus, acetone and diacetic acid. The blood sugar was 530 mg. per cent and the van Slyke 14 volumes per cent. The red cell count and hemoglobin were normal and the leukocyte count 13,600. On December 22nd, after insulin, the urine was sugar-free, the blood sugar 60 mg. per cent, the blood urea nitrogen 17 mg. per cent, and the van Slyke 25 volumes per cent. The phenolsulphonephthalein excretion on this day was 58 per cent in 2 hours. On December 23rd the urine showed sugar 1 plus, albumin 3 plus, many casts and acetone, and the blood sugar was 350 mg. per cent. On December 24th the patient developed anuria, the blood urea nitrogen was 67 mg. per cent, and the van Slyke 9 volumes per cent. Very little urine was voided after December 23rd. On December 25th the blood pressure was 140/66. Death occurred on December 25th.

Blood taken after death showed urea nitrogen 123 mg. per cent and creatinine 5 mg. per cent. There was no subcutaneous edema, but the peritoneal cavity contained 300 cc. and the pleural cavities 600 cc. of clear fluid. There was terminal bronchopneumonia. The kidneys were greatly enlarged and very pale, weighing 240 gm. and 220 gm. each. Microscopically all the tubules are enormously dilated and lined by thin, eosin-staining cells (Fig. 14). There is no glomerulitis and there are only a few casts. The diagnosis is extrarenal uremia with renal injury.

(*B*) *Without Renal Injury*

In this group of cases there is marked renal insufficiency but the kidneys are practically normal on both macroscopic and histological examination.

21. 34–194. *Diabetic coma.* A negress, 63 years of age, was admitted to the hospital in coma on January 28th. On the day of admission she had had several convulsions, during which she became unconscious. She had been unconscious 3 hours prior to admission. The blood pressure was 152/90. The hemoglobin and red cell count were normal, and the leukocyte count was 31,200. The patient was

incontinent and no urine was obtained for examination. Death occurred on the morning of January 31st.

A postmortem sample of blood showed blood sugar 600 mg. per cent, urea nitrogen 89.6 mg. per cent and creatinine 4.2 mg. per cent. Postmortem examination revealed an extensive bronchopneumonia and a small carcinoma of the head of the pancreas. The kidneys weighed 100 gm. and 140 gm. each. There were cortical scars from atherosclerosis. Microscopically the kidneys are normal.

22. 34–219. *Gangrenous pancreatitis.* A male, 68 years of age, developed a sudden attack of abdominal pain on the morning of January 20th. He vomited twice that afternoon. He was admitted to the hospital on the afternoon of January 20th, at which time his entire abdomen was distended and tympanitic. There was evidence of paralytic ileus. He was given fluids intravenously and nasal suction. His general condition seemed to improve but he continued to vomit occasionally. Five specimens of urine were examined, two of which showed a trace of albumin while the others were normal. The leukocyte count ranged from 14,650 to 19,100. On January 27th the blood urea nitrogen was 86.8 mg. per cent and creatinine 2.5 mg. per cent. Death occurred January 28th.

The principal findings at postmortem were gangrenous pancreatitis and cholelithiasis. The kidneys were enlarged, weighing 219 gm. and 242 gm., but on microscopic examination they appear entirely normal.

23. 36–1055. *Gangrenous pancreatitis.* A male, 45 years of age, developed an attack of acute abdominal pain on May 22nd and was brought to the hospital. There was nausea but no vomiting. On the same day the gall-bladder and appendix were removed; the gall-bladder showed an inactive old cholecystitis and was filled with stones. He was given intravenous glucose and nasal suction. He perspired profusely almost constantly and vomited occasionally. A specimen of urine on May 25th showed albumin 3 plus, and granular casts, and the blood urea nitrogen was 53 mg. per cent. There is no record of the diuresis. The patient gradually became stuporous and irrational. On May 29th the blood urea nitrogen was 142 mg. per cent and creatinine 8.8 mg. per cent. Muscular twitchings all over the body were noted on May 30th and May 31st. Death occurred June 1st. Postmortem examination revealed acute gangrenous pancreatitis and generalized peritonitis. The kidneys weighed 150 gm.

and 175 gm. and were normal on gross and microscopic examination.

24. 34–481. *Paralytic ileus.* A young male, 19 years of age, sustained a fracture of the second lumbar vertebra on February 20th. He was in good condition until March 6th when he began to vomit and ceased to have bowel movements. On March 10th his abdomen was greatly distended. The urine on this day showed a specific gravity of 1020 and no albumin. He vomited continuously after March 6th. The blood pressure remained about 106/70. He was given large amounts of intravenous saline solution. Death occurred March 14. A postmortem sample of blood showed urea nitrogen 134 mg. per cent; creatinine 6.85 mg.; non-protein nitrogen 313 mg.; and total blood chloride 460 mg. Postmortem examination revealed a paralytic ileus. The kidneys weighed 170 gm. and 200 gm., and show no gross or microscopic evidence of disease.

25. 34–548. *Primary peritonitis.* A male, 62 years of age, developed primary peritonitis and died 8 days after the onset of symptoms. He vomited continuously from the onset of his illness. He was under observation only during the last day of his life. A small amount of urine was obtained by catheter. The specific gravity of the urine was 1020, albumin 2 plus, and many granular casts were present. The blood urea nitrogen was 109 mg. per cent. The blood pressure was 100/60.

On postmortem examination the peritoneal cavity contained 1500 cc. of purulent fluid. The kidneys weighed 140 gm. and 130 gm. Microscopically the kidneys are practically normal.

26. 34–929. *Streptococcic infection.* A male, 71 years of age, was admitted to the hospital on May 19th. On May 14th he had noted an infection of the right ankle, which spread over his entire leg during the next 2 days. The right inguinal nodes became enlarged and tender. On admission his temperature was 101.6° F. and his blood pressure 116/40. The urine showed a specific gravity of 1009 and a trace of albumin. On May 24th the urine showed a specific gravity of 1017 and albumin 1 plus; the leukocyte count was 37,000; the blood urea nitrogen was 156 mg. per cent and creatinine 5 mg. Death occurred May 24th.

On postmortem examination a severe hemolytic streptococcic infection of the leg was noted. The kidneys weighed 170 gm. and 200 gm. and their cortices were pale. Microscopically the kidneys are normal.

27. 35–2028. *Suppurative cholangitis.* A female, 51 years of age, was admitted to the hospital December 13th, complaining of upper abdominal pain, nausea and vomiting. These symptoms had been present for about 3 weeks. She was somewhat stuporous and moderately jaundiced. The blood pressure was 88/50. The urine contained bile but no albumin or sugar. The icterus index was 39. The hemoglobin was 72 per cent; erythrocytes, 3,750,000; leukocytes, 13,400. The blood urea nitrogen was 101 mg. per cent. The patient gradually became more stuporous and died on December 15th.

Postmortem examination revealed suppurative cholangitis with multiple abscesses in the liver and the lungs. The kidneys weighed 130 gm. and 140 gm. and their cortices were cloudy. Microscopically the kidneys are normal.

"Hypochloremic" Uremia

The best known form of extrarenal uremia is that associated with intestinal obstruction. It has long been known that intestinal obstruction gives rise to oliguria, an increase of nitrogenous waste products in the blood, a decrease of blood chlorides and often clinical uremia. It has been shown both clinically and experimentally that injection of a physiological solution of sodium chloride increases the diuresis, relieves the clinical symptoms, decreases the blood urea and non-protein nitrogen and increases the blood chlorides. In fatal cases it has been found that the kidneys are structurally normal. The unfavorable symptoms have generally been referred to the low level of blood chloride; chlorides are lost by the excessive vomiting which commonly accompanies the disease. The renal insufficiency associated with intestinal obstruction is usually called hypochloremic uremia. The chief argument that low blood chlorides are responsible for the uremia is the improvement that follows administration of sodium chloride. Many investigators have tried to explain other forms of extrarenal uremia on the basis of low blood chlorides. Blum and his associates (1929) advanced the theory that urea and other metabolites are retained, when the blood chlorides decrease, in order to maintain the osmotic pressure of the blood.

In recent years, however, convincing evidence has accumulated which shows that the level of the blood chlorides has no causal relation to the level of blood urea or non-protein nitrogen. Dehydra-

tion and increased protein metabolism appear to be the chief causes of extrarenal uremia.

Schiff (1929) gave an extensive survey of the literature on dehydration. He noted that when the water supply of an infant is decreased without diminishing the other constituents of the milk, the child develops toxic symptoms and the non-protein nitrogen of the blood is markedly increased. The plasma chloride is also increased. In pups a low water intake with a normal protein intake causes similar changes and leads to fatty degeneration of the liver, but a low water intake with a protein-free diet does not cause any serious disturbances and there is no increase of non-protein nitrogen in the blood. Kerpel-Fronius (1932) cited a clinical case of dehydration in which the serum chloride was 514 mg. per cent and the non-protein nitrogen 165 mg. per cent. He thinks that azotemia in cases of this type is related to oliguria and dehydration but not to the blood chloride.

Mach and his associates (1934) reported 3 cases of cirrhosis of the liver with marked ascites. The ascitic fluid was removed repeatedly in large amounts, in one patient 92 liters were removed during a period of 7 months. The ascitic fluid had a constant content of chloride, about 600 mg. per cent. During this time the plasma chlorides sank to low levels, 270 to 350 mg. per cent, but the blood urea was not increased in any instance. No better example than this could be cited to show that hypochloremia does not cause azotemia.

Grünwald (1909) found that repeated injections of diuretin in rabbits caused excessive loss of chloride in the urine and that the animals died in coma with a low blood chloride. Bilbao and Grabar (1929) observed that a high blood urea developed along with the low blood chloride but that if salt solution was supplied the animals did not develop azotemia. These investigators attributed the azotemia to hypochloremia. Kerpel-Fronius and Butler (1935) repeated these experiments with diuretin and observed a concentration of the blood as indicated by the increase of the plasma proteins. They also found that administration of water without salt prevented both the azotemia and the concentration of the blood. They attributed the beneficial effects of salt solution to the increased diuresis and not to the effect on the serum electrolytes.

Kerpel-Fronius (1936) removed a large proportion of the chlorine

ions from the blood by intraperitoneal injections of sodium lactate; the sodium ion was not removed. In this experiment the non-protein nitrogen of the blood was not increased. This author gives a summary of the evidence against the view that low blood chloride is a cause of azotemia.

In 5 of our cases (Nos. 17, 18, 22, 24, 25) there was excessive loss of fluid by vomiting, and in No. 23 excessive perspiration probably caused a depletion of body fluid. Although the blood chlorides were not determined these cases presumably belong to the group of so-called hypochloremic uremia. In 4 of these cases the kidneys were normal but in two instances, Nos. 17 and 18, the kidneys were greatly swollen and there is evidently some tubular injury. However, the tubular injury does not seem severe enough to have caused uremia and we must believe that loss of body fluid was chiefly responsible for the decreased urinary output and subsequent retention of nitrogenous products.

In 5 cases (Nos. 13, 14, 16, 26, 27) there was no excessive loss of body fluid although there may have been a decreased fluid intake. There was a severe infection in each of these patients and in the first three definite tubular injury was found at postmortem. However, the structural changes seem too mild to have produced uremia. It is possible that increased destruction of tissue protein, together with an inadequate fluid intake, is responsible for the uremia.

The Uremia of Diabetic Coma

Many investigators have noted that uremia may develop during or after an attack of diabetic coma. Severe oliguria or anuria begins usually 24 to 48 hours after the onset of coma. The patient frequently recovers from coma before the onset of anuria. In 54 cases of diabetic coma Baker found the blood urea nitrogen above 35 mg. per cent in 27; in 15 cases it was above 50 mg. per cent, and in 3 cases above 100 mg. per cent. In a survey of the literature of diabetic coma Fullerton *et al.*, found 6 deaths among 34 patients in whom the blood urea value did not exceed 100 mg. per cent, but there were 14 deaths among 22 in whom the blood urea was above this level.

The azotemia is obviously related to urinary suppression; the problem under discussion for some years is the cause of the decreased diuresis. The urine usually contains a fairly large amount of albumin but this does not indicate a serious renal injury; albumin escapes

through glomerular capillaries and these show no structural changes. If any serious renal injury is present it must be tubular. During the stage of oliguria and nitrogen retention the kidneys can secrete little or no phenolsulphonephthalein, which may mean decreased glomerular filtrate or increased tubular reabsorption.

Several observers have noted that the kidneys are enlarged and cloudy (Warburg, Fullerton *et al.*, Dinkin and Metzger, Metzger, Kraus and Selye); and several have described microscopic tubular lesions, such as granular and vacuolar degeneration and occasional necrotic cells, and have considered the anuria a result of tubular injury (Labbé and Boulin, Fullerton *et al.*, Kraus and Selye). The injury of the kidneys is usually attributed to ketonic acids (Snapper). Metzger studied 17 cases and observed some renal injury but does not think the damage sufficient to cause uremia.

In 2 of our cases the kidneys were greatly enlarged; in 1 of them (No. 19) there was no change but a moderate dilatation of the tubules (Fig. 13), but in the other (No. 20) there was an enormous tubular dilatation (Fig. 14). There is no obstruction in the tubules to explain the dilatation. In this particular case (No. 20, Fig. 14) one is forced to conclude that tubular injury is partly responsible for the azotemia. In our 3rd case (No. 21) the kidneys were entirely normal microscopically. In summary we may say that there is usually some tubular injury in diabetic coma with uremia but that it is seldom sufficiently severe to explain the uremia.

The blood chlorides are sometimes moderately reduced, sometimes normal (Labbé and Boulin, Fullerton *et al.*, Blum *et al.*, Schmitt). There is no evidence in the literature that the uremia of diabetic coma is of the hypochloremic type.

Bulger and Peters found a definite concentration of the blood in diabetic coma, indicated by the increase of hemoglobin and plasma proteins.

The cause of anuria and azotemia in diabetic coma is not clearly established. There is evidence that the following factors may be concerned, *viz.* dehydration, increased endogenous protein metabolism, acidosis with injury of the renal tubules and decreased blood pressure.

Posthemorrhagic Uremia

Meyler (1935) observed 2 clinical cases of uremia following severe hemorrhage. He reproduced this condition in guinea pigs by re-

peated bleedings. The blood urea rose to high levels but the blood chloride remained at a normal level. The patients as well as the animals secreted a small amount of highly concentrated urine. The animals took very little fluid or food. When given fluid subcutaneously the guinea pigs did not develop uremia. Meyler thinks that there is an enormous destruction of body protein and that the kidneys are unable to excrete the increased amount of nitrogenous waste products because of dehydration.

Cerebral Uremia

It is now well known that various types of injury of the brain may give rise to transitory albuminuria and glycosuria (Morawitz and Schloss). It is seen most frequently after subarachnoid hemorrhage. The albumin and sugar are noted directly after the injury and often both disappear within 48 hours. Acetone is seldom found. Occasionally there is anuria. Morawitz and Schloss reported one patient with a non-protein nitrogen of 134 mg. per cent and another with 162 mg. per cent; both recovered.

We have observed one patient, admitted in coma from carbon monoxide poisoning, who showed albumin and a large amount of sugar in the urine. The condition was diagnosed diabetic coma at first. The postmortem revealed normal kidneys. Another patient who sustained a traumatic injury of the brain was first seen in coma with albuminuria and glycosuria, but there was no acetonuria. The postmortem examination revealed bruising of the frontal lobes of the brain but no subarachnoid hemorrhage. The kidneys were normal.

A third patient with a subarachnoid hemorrhage developed a non-protein nitrogen of 160 mg. per cent but subsequently recovered.

Cerebral glycosuria is attributed to injury of the sugar-regulating center in the floor of the fourth ventricle. There is no satisfactory explanation for cerebral albuminuria and uremia. It has been suggested that uremia is due to oliguria induced by spasm of the renal vessels. Low blood pressure is sometimes a possible factor but azotemia may develop with a normal or an increased blood pressure.

In summary it may be said that several factors may be concerned in the development of extrarenal uremia, the most important of which are dehydration and increased endogenous protein metabolism. It is unlikely that a decrease of blood chlorides is a cause of azotemia.

Summary

One hundred and ten cases of clinical acute nephritis have been classified in accordance with the structural changes in the kidneys.

There were 31 cases of uncomplicated acute glomerulonephritis and 20 cases in which the nephritis was associated with another disease.

Obstruction of the glomerular circulation is usually due to endothelial proliferation, but in a few instances it is due partly or largely to epithelial crescents, intracapillary thromboses, thrombosed arterioles or polymorphonuclear leukocytes.

In the normal glomerulus and in subclinical glomerulonephritis it may be seen that all the capillaries of the lobules are completely invested with a basement membrane, but in clinical glomerulonephritis the capillaries within the lobule become fused together and their inner basement membranes split to form the characteristic intracapillary fibers. The lesions are all intracapillary; the appearance of "intercapillary" lesions is due to the persistence of portions of the capillary lumens in the peripheral parts of the affected lobules.

In 5 cases uremia was due to numerous massive lesions of the embolic type, in the absence of endocarditis.

Eleven cases are reported in which the outstanding symptoms were septicemia, hematuria and uremia. This is called the hemorrhagic type of glomerulonephritis. The blood escapes through ruptured glomerular capillaries and uremia is due to obstruction of the tubules by masses of red blood cells or hemoglobin.

Albuminuria, hematuria and edema of renal origin are evidences of glomerular injury; tubular disease is evidenced by oliguria and anuria.

In rare instances acute uremia is due to multiple thromboses of small renal arteries.

The most frequent form of tubular nephritis is that associated with mercuric chloride poisoning but there are occasional instances of tubular disease due to other causes.

The acute uremia following transfusion with incompatible blood is due chiefly to obstruction of the collecting tubules by casts of hemoglobin.

There is a group of cases in which uremia seems to be partly of extrarenal origin and partly due to distention of the convoluted tubules with minor degenerative changes in their lining cells.

In purely extrarenal uremia the kidneys are normal and the azotemia is due chiefly to dehydration and to increased destruction of protein. Decrease of blood chloride is apparently not a cause of azotemia.

The azotemia of diabetic coma is due in part to tubular injury in some instances.

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DESCRIPTION OF PLATES

PLATE 85

Fig. 1. (Table I, No. 3.) Lobule of a glomerulus showing an early stage of acute proliferative glomerulonephritis. The outer basement membrane (o b m) of the capillaries is intact but the inner basement membrane (i b m) has begun to break up into intracapillary fibers.

End. = endothelial cell; ep. = epithelial cell; er. = erythrocyte. Mallory-Heidenhain stain. × 1200.

FIG. 2. (Table I, No. 10.) Lobule of a glomerulus showing a more advanced stage of acute proliferative glomerulonephritis than that illustrated in Figure. 1. The capillaries composing the lobule are fused into a compact mass. The inner basement membranes have broken up into intracapillary fibers (f). Other lettering as in Figure 1. Mallory-Heidenhain stain. × 1200.

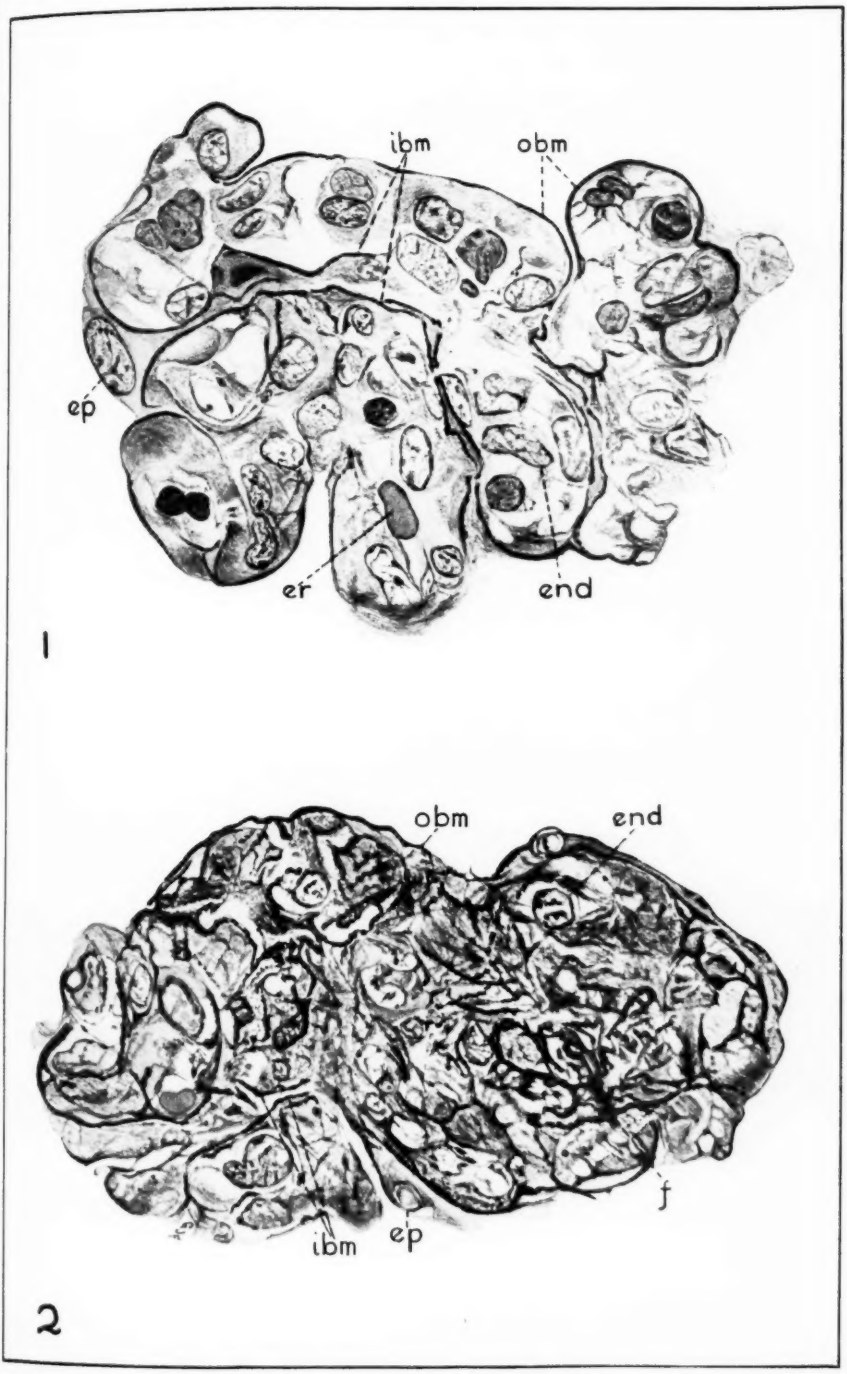


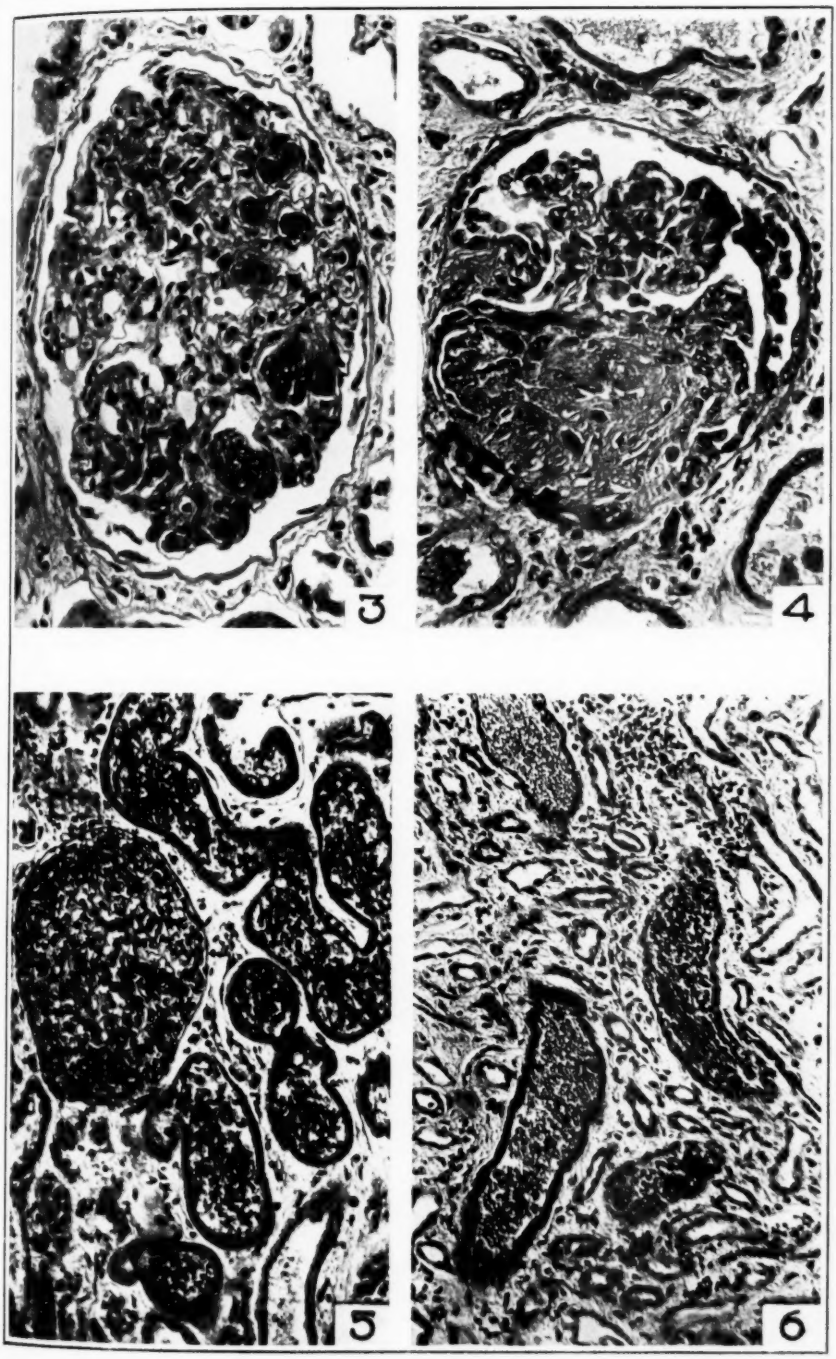
PLATE 86

FIG. 3. (Table I, No. 52.) Glomerulus showing thrombosis of nearly all of its capillaries. × 350.

FIG. 4. (Table I, No. 64.) Glomerulus showing an embolic type of lesion. Nearly all the glomeruli showed lesions similar to this. There was no endocarditis. × 350.

FIG. 5. (Table I, No. 75.) Hemorrhagic type of glomerulonephritis. Note that the capsular space and the tubules are distended with blood. × 250.

FIG. 6. (Table I, No. 72.) Hemorrhagic type of glomerulonephritis. Note collecting tubules distended with casts composed largely of hemoglobin. × 300.



Bell

Clinical Acute Nephritis

PLATE 87

FIG. 7. (Table I, No. 4.) From a case of mercuric chloride poisoning of 10 days duration. Note that the cells of some of the convoluted tubules are largely destroyed; only a thin basal zone of cytoplasm persists. × 400.

FIG. 8. (Table I, No. 7.) From a case of mercuric chloride poisoning of 16 days duration. Note that the lining cells of the convoluted tubules are flattened, and that they take the basic stain. The normal granulation is absent. These are interpreted as regenerated epithelial cells. × 350.

FIG. 9. (Table I, No. 9.) Tubular nephritis. Note the extreme hydropic degeneration of the convoluted tubules. × 350.

FIG. 10. (Table I, No. 11.) Obstructive tubular disease from blood transfusion. Area from the cortex showing dilation of tubules and interstitial edema. × 200.

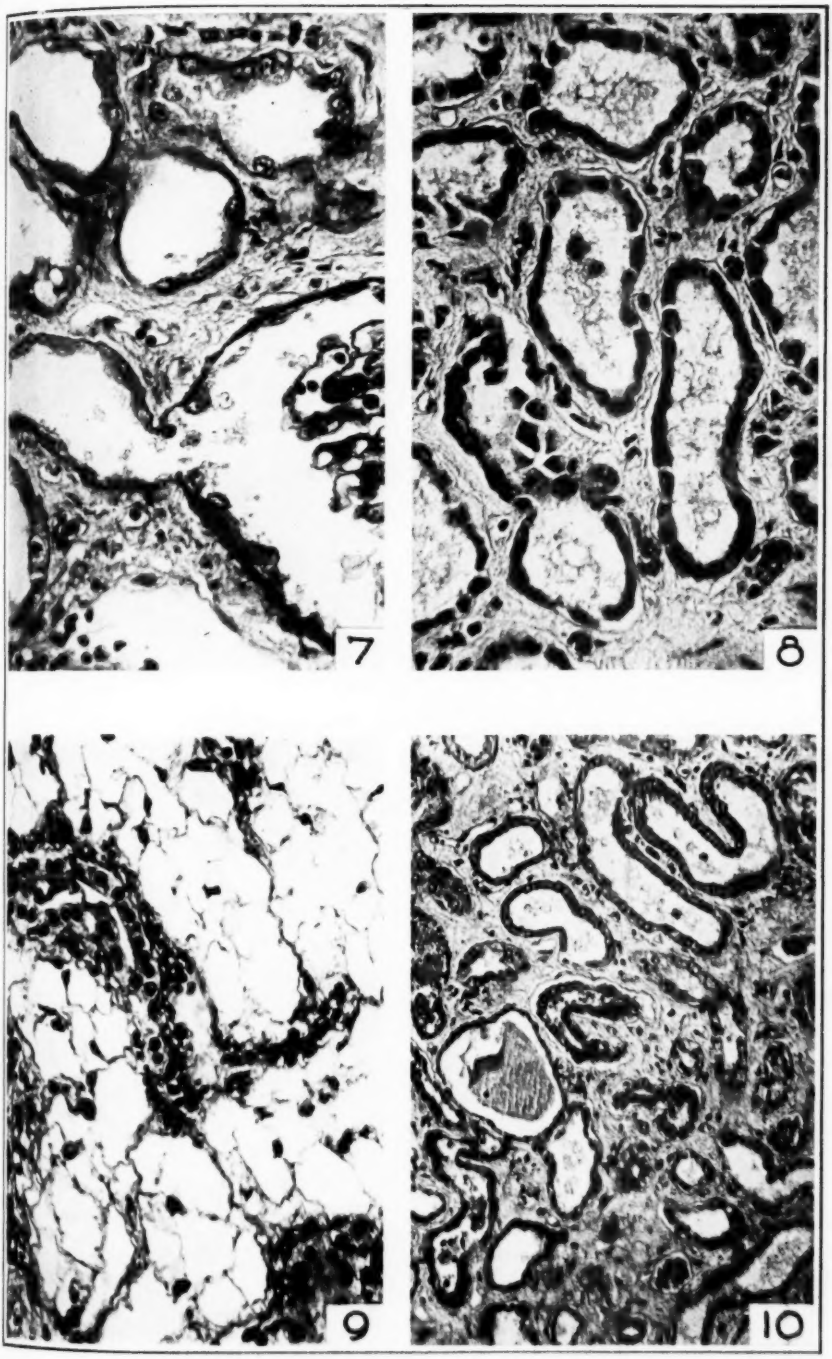

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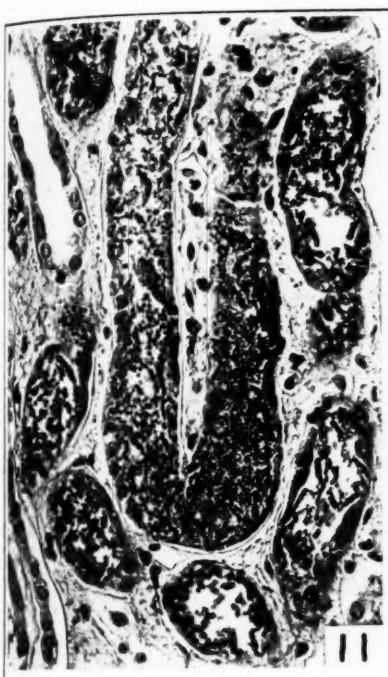
PLATE 88

FIG. 11. (Table I, No. 11.) Obstructive tubular disease from blood transfusion. Area from the medulla showing collecting tubules obstructed by casts composed of hemoglobin. × 350.

FIG. 12. (Table I, No. 14.) Extrarenal uremia with some tubular injury. The cells of the convoluted tubules are filled with small vacuoles but there are no necrotic cells. × 450.

FIG. 13. (Table I, No. 10.) Extrarenal uremia with some tubular injury. The kidneys weighed together 600 gm. All the cortical tubules are dilated but only a few casts are found. There is no necrosis. × 350.

FIG. 14. (Table I, No. 20.) Extrarenal uremia with some tubular injury. Death from diabetic uremia. The kidneys weighed together 460 gm. All the tubules are enormously dilated and lined by pale flattened epithelium. No definite necrosis is seen. There are only a few casts. × 350.



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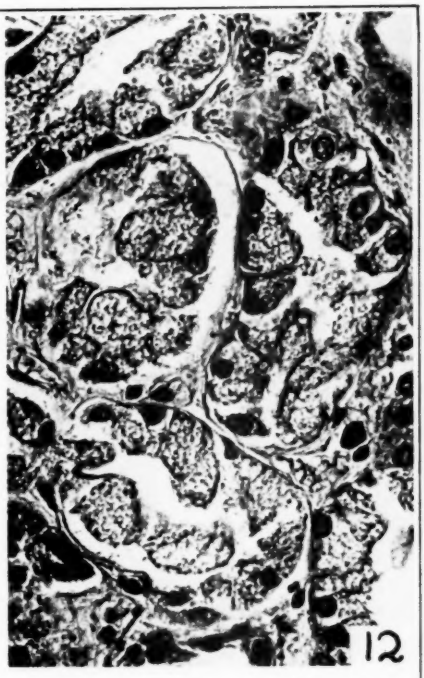

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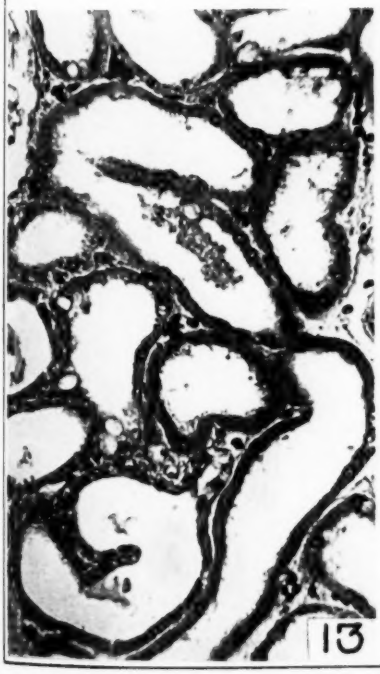

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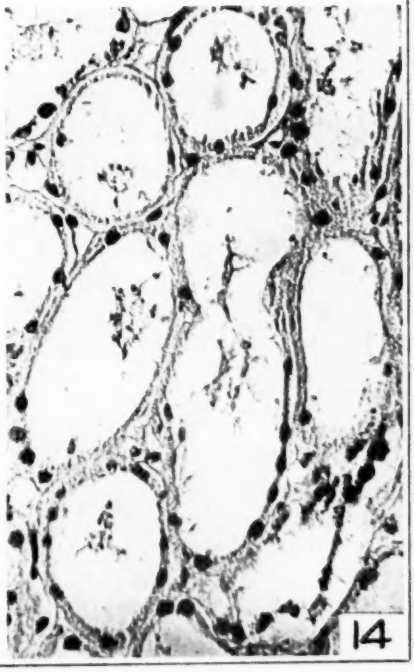

14

PARATHYROID HYPERPLASIA IN CHRONIC RENAL INSUFFICIENCY *

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In contrast with the present universal recognition of the effects of parathyroid abnormalities on the skeletal system relatively little attention has been devoted to the close interrelation of the kidneys and the parathyroid glands. Yet there is reason to believe that instances of the latter are far commoner than the former. For this relation appears to work in either direction. Hyperparathyroidism, if sufficiently long continued, will eventually lead to renal insufficiency because of calcium deposits in the kidney parenchyma, while primary renal insufficiency of a severe and prolonged character will produce parathyroid hyperplasia and — if we accept as evidence the histological alterations in the bones and Shelling and Remsen's [1] parathormone assay of the blood — true functional hyperparathyroidism. Starting, therefore, with either primary lesion a patient may reach an apparently similar end-stage of combined hyperparathyroidism and renal insufficiency. The possibility, therefore, of distinguishing between primary and secondary changes in the parathyroid gland has practical as well as theoretical significance.

In a previous paper describing the pathology of the parathyroid glands in 25 cases of hyperparathyroidism [2] a classification was proposed that divided the lesions sharply into two groups, one in which the changes were restricted to one gland, part of a gland, or occasionally two glands (presumably neoplasia), and a second group in which diffuse hypertrophy and essentially uniform histological changes in all the glands occurred — a condition we classified as hyperplasia. We described furthermore two types of hyperplasia; one in which all the cells are unusually large with clear cytoplasm and nuclei uniformly oriented to the base of the cell (true wasserhelle cells), and another type in which all the glands are composed, except for a scattering of oxyphil cells, of closely packed, normal sized chief cells. A single case of this chief cell type of hyperplasia was

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presented and though it was recorded as being found in a patient with chronic renal insufficiency this relation was not stressed.

In the two and a half year period since our former report 12 new cases of hyperparathyroidism have been seen at the Massachusetts General Hospital. Ten of these were single adenomas, 1 was a wasserhelle hyperplasia, and 1 a chief cell hyperplasia again associated with a long-standing renal insufficiency. No case has been seen that failed to fit readily into the previously described categories and the additional clinical evidence that has accumulated — the absence of a single recurrence of symptoms in the adenomatous group contrasted with several recurrences in the hyperplastic group — strongly substantiates the validity of our classification.

The observation of a second case of pronounced chief cell hyperplasia in association with osteitis fibrosa and chronic renal insufficiency served to emphasize the distinction between the wasserhelle type of hyperplasia — to be called hereafter for the sake of simplicity "primary hyperplasia" even though we realize that it cannot be truly a primary disease of the parathyroids — and the entirely different hyperplasia that is secondary to chronic renal insufficiency. This stimulus led us to study the parathyroids in a group of 29 cases of chronic renal insufficiency and also to search for secondary hyperplasia in other disease states. The present report is a résumé of this study. Our material consists of 2 cases (1 previously reported) of chronic renal insufficiency with bone lesions indistinguishable from those of hyperparathyroidism, 12 cases of chronic glomerular nephritis without bone lesions, 15 cases of renal insufficiency of other types, and an assortment of 9 other cases in which secondary hyperplasia was found. We have included also for purposes of contrast and because cases of this type are still not numerous an additional case of so-called primary hyperplasia.

Review of the Literature

The fact that one or more parathyroid glands may be enlarged in chronic glomerular nephritis or in any form of chronic renal insufficiency is not an original observation. In a paper published in 1935 Pappenheimer and Wilens [3] compared the weights of the parathyroids of normal individuals with those of a group of nephritics and showed quite conclusively that the latter were 50 to 100 per cent heavier. In a later communication Jarrett, Peters and Pappen-

heimer [4] reported the production of enlargement of the parathyroids in rats by a total nephrectomy on one side and partial cauterization of the other kidney. They did not, however, discuss the histological changes in the glands and descriptions of the latter are comparatively rare.

More recently Gilmour and Martin [5] published an exhaustive statistical study of the weights of the parathyroids in a series of 527 cases of varied diseases. They calculated the weight of the parenchyma as distinct from the fatty stroma and from their figures the parenchymatous portions of the glands from the nephritics and renal disease groups were over 60 per cent heavier than the normals. Many of the cases included in their renal group, however, were probably not cases of chronic renal insufficiency so that this figure would certainly have been much higher had cases of the latter only been selected. Here also no histological studies were reported.

In 1905 MacCallum [6] reported a case of chronic glomerular nephritis in which he found one enlarged parathyroid and two apparently normal glands. Bergstrand [7] in 1921 reported 10 cases of parathyroid enlargement associated with chronic renal insufficiency. In 7 of these cases there was a diffuse chief cell hyperplasia of all the glands. In some the gross enlargement was especially marked in one or two of the glands of an individual case but similar histological changes were present in all of the glands. The remaining 3 cases showed enlargement of only one gland, the others being normal in size and structure. In one of this group a rim of normal parathyroid tissue about an apparent adenoma was described. In none of these cases were any clinical data recorded and there was no mention of examination of the bones beyond the statement that one of the cases showed osteoporosis.

The numerous case reports of renal rickets — a disease of children characterized by chronic renal failure usually due to an anomaly in the urinary tract with secondary demineralization of the skeleton — might be expected to provide considerable information relative to the parathyroids. Unfortunately, however, they have not been examined or at any rate described in most of the cases of renal rickets that have been reported. Mitchell [8] in 1930 abstracted 78 cases of renal rickets but makes no mention of the parathyroids in any of them. We have checked most of these case reports and found that many were purely clinical and that in those where autopsies

had been performed examination of the parathyroids was not recorded.

Langmead and Orr[9] in 1933, Smyth and Goldman[10] in 1934, and Price and Davie[11] in 1937 reported cases of renal rickets with diffuse parathyroid enlargement. In Hubbard and Wentworth's[12] case of metastatic calcification and osteitis fibrosa associated with chronic nephritis two parathyroid glands were identified and were said to be hyperplastic though no microphotographs or microscopic descriptions were given.

Shelling and Remsen[1] reported a case of renal rickets in which increased amounts of parathyroid hormone were demonstrated in the blood and in which four enlarged parathyroid glands were found. The description and microphotographs of the glands are identical with our cases of secondary hyperplasia.

Cases of Parathyroid Hyperplasia

A. Primary Hyperplasia

Case 26*: A. T. (35–534), a housewife, aged 57 years, was admitted Jan. 21, 1935. Following three attacks of renal colic she passed a urinary calculus in March, 1931. One month later her right kidney was removed at another hospital. In the summer of 1934 she experienced almost daily attacks of dull pain in the left lower back radiating at times to the groin. During the 6 months prior to admission she had vague aches and pains throughout her body and had lost 15 pounds. Her weight on admission was 83 pounds.

X-rays showed generalized skeletal decalcification with several areas of diminished density in the skull that suggested multiple myeloma. The serum calcium ranged between 14.46 and 16.38 mg. per cent, and the serum phosphorus from 2 to 2.91 mg. per cent. The phosphatase was 4.84 Bodansky units. A bone biopsy showed the changes characteristic of hyperparathyroidism. Renal function tests showed normal excretion by the remaining kidney.

On Feb. 9, 1935, the parathyroid glands were exposed and found to be enlarged (Fig. 1). Both superior and the right inferior glands were entirely removed. Approximately three-fourths of the left lower gland was resected, leaving residual tissue estimated at 0.225 mg. with a good blood supply.

Gross Description: (Left Upper): A somewhat flattened, soft, triangular shaped, slightly fluctuant mass 5 by 2 by 3.5 cm. which weighed approximately 6 gm. The surface of the upper two-thirds was purplish red, while that of the lower third was yellowish brown. On section the upper two-thirds was made up of many small cystic

* This is the 26th case in the Massachusetts General Hospital series and the number is used here to correspond with any reference to it in previous or subsequent articles.

cavities 1 to 3 mm. in diameter, from which dark red blood exuded. The lower third was homogeneously yellowish brown.

(*Left Lower*): A smooth surfaced, yellowish brown, encapsulated soft mass measuring 1.5 by 1 by 0.8 cm. which weighed 0.86 gm. The cut surface was uniformly yellowish brown. A portion of this gland was not resected.

(*Right Upper*): An irregularly shaped, encapsulated, lobulated, soft, smooth surfaced mass which measured roughly 3 by 2 by 1 cm. and weighed 3.75 gm. The surface of some parts of the gland was dark purplish red and other parts yellowish brown. One pole was composed of three pseudopod-like projections 1.5, 1 and 1 cm. in length and approximately 1.5 to 1 cm. in diameter. The cut surface was reddish to yellowish brown.

(*Right Lower*): Similar to the left lower, measured 1.8 by 0.7 by 0.3 cm. and weighed 0.59 gm.

Microscopic Examination: A detailed description of this type of hyperplasia was given in our previous paper * but is so perfectly applicable to this case that it will be repeated here in order to compare it with the hyperplasia secondary to chronic renal insufficiency. All the glands have the same appearance. "There is only one type of cell throughout, the wasserhelle cell, which is polyhedral in shape, sharply demarcated by a thin eosinophilic membrane, and varies from 10 to 40 μ in diameter, averaging 15 to 20 (Fig. 3). Many of the cell boundaries are broken, with resultant fusion, similar to the fusion of alveoli in pulmonary emphysema. In contrast with the variability in the size of the cells, the nuclei, though often multiple, are all approximately the same size, averaging about 8 μ in diameter. They are round to slightly ovoid in shape, sharply outlined, moderately hyperchromatic, with an eccentrically placed nucleolus. As a rule the nuclei are located in the end of the cell that is contiguous to the stroma. This produces a characteristic pattern which resembles branches of berries (Fig. 2). The cytoplasm is clear except for a little, light pink-staining granular material. Many of these tiny granules are glycogen deposits. Similar granules are present within the nuclei. There is no fat, except for a rare droplet in the stroma. The low power appearance of the histological sections is so similar to that of clear cell renal carcinomas that distinction would be difficult if the source were not known. The stroma is composed of thin,

* Case 15, pages 7 and 8.

fibrous connective tissue bands containing a moderate number of connective tissue cells and relatively few blood vessels. These bands surround small and large groups of cells, producing a pseudo-glandular effect. This effect is further emphasized by the position of the nuclei, as mentioned above. Occasionally a true single layered alveolus is seen. No oxyphil or chief cells are found. There are no mitoses."

B. Hyperplasia, Secondary to Chronic Renal Insufficiency

Group 1. With Secondary Osteitis Fibrosa and Ectopic Calcification *

F. L. S. (7964)†, a 45 year old American, entered the hospital on Nov. 5, 1935, complaining of painful nodules in his fingers. Twenty-three years before admission he was studied at another hospital for generalized dropsy, was told he had incurable Bright's disease, and was placed on a salt-free, meatless diet. From that time until 2½ years before admission the patient was in apparent good health with no return of edema. Then he began to have itching of the skin, nocturia, and later swelling of his fingers. The positive findings in the physical examination were precordial systolic and diastolic murmurs, cystic swellings on the right forefinger, and firm and tortuous peripheral vessels. The blood pressure was 165/90.

Examination of the urine showed a specific gravity from 1.006 to 1.012, a large trace of albumin and a sediment containing an occasional red blood cell and white blood cell. A phenolsulphonephthalein test showed less than 15 per cent excretion at the end of 1 hour. Examination of the blood showed a red blood cell count of 3,800,000 with a hemoglobin of 65 per cent, and a white blood cell count of 12,000, 63 per cent polymorphonuclears. The non-protein nitrogen was 120 mg. The serum calcium was 10.10 mg. per cent, serum phosphorus 7.92 mg. per cent, and phosphatase 9.36 Bodansky units. The serum protein was 4.9 gm. per cent. X-ray examination showed masses of homogeneous calcification surrounding the proximal interphalangeal joints and along the phalanges of the right second, third and fourth fingers. Both elbows and the acromioclavicular joints showed similar calcified masses. The bones of the pelvis showed slight decalcification. The skull was riddled with small areas of decalcification. In all the films the large and small blood vessels showed marked arteriosclerosis with calcification, some of them definitely of the Mönckeberg type. A pyelogram showed extremely small kidneys.

While on the ward during the 3rd week he was suddenly seized with severe pain between the shoulder blades and later the pain was localized in the left anterior chest and upper abdomen. He developed bundle branch block; the blood pressure fell to 60/50 and he died 5 hours later.

* One similar case was reported in our previous paper,[2] Case 23A, page 12.

† A clinical discussion of this case has been reported separately by Dr. Fuller Albright in the *Tr. A. Am. Physicians*, 1936, **51**, 199–212. This case has also been reported in the Case Records of the Massachusetts General Hospital, *New England J. Med.*, 1936, **214**, 320–325.

Postmortem examination showed in addition to the parathyroid enlargement a marked chronic glomerular nephritis, metastatic calcification around joints, calcification of the coronary, renal and splenic arteries, coronary occlusion, and rheumatic heart disease with mitral stenosis. Death was due to coronary thrombosis. Sections of the various bones showed a mild to moderate degree of decalcification and osteitis fibrosa (Fig. 9). The latter was indistinguishable from the bone lesions in primary hyperparathyroidism, except that no cysts were present.

Gross Description: All four parathyroid glands were in their normal position. They were all enlarged, firm, yellowish white, and somewhat lobulated (Fig. 4). The cut surface was uniformly yellowish white and smooth. (Note the absence of any brown color, a finding that is present in normal glands, adenomas, and primary hyperplasias.) In one gland a calcified nodule was found. The right lower was the largest, measured 2 by 1.5 by 1.5 cm., and one-half of it weighed 2 gm. From the weight of this half the weights of the glands were estimated as: right lower 4 gm., left lower 3 gm., and the upper glands 2 gm. each.

Microscopic Examination: The picture here is almost identical with that of the case of chief cell hyperplasia described in our previous paper. None of the architectural features of a normal parathyroid gland can be made out. Instead of anastomosing columns of epithelial cells separated by bands of fibrous stroma containing large fat cells, the tissue consists of an almost solid sheet of epithelial cells punctuated only by vascular channels at rather regular intervals with very scant fibrous stroma chiefly limited to the adventitia of the blood vessels (Fig. 8). Practically no fat cells are present. The structure is by no means uniform, however, for even with low power it is evident that there are circumscribed islands of varying size often encapsulated with a delicate band of collagen. Either a peculiarity of the arrangement of the cell cords or a consistent variation in the cells themselves serves to mark the islands clearly from the surrounding tissue (Figs. 5 and 6). Throughout the great majority of the gland the predominant cell is the chief cell, a trifle more vacuolated than normal, but with a cytoplasm by no means totally clear. Within each of the circumscribed islands which have been mentioned the cells tend to be very similar in appearance but between one island and another there may be marked variation

(Fig. 7). In one, for instance, the cells are uniformly large and tend to show a high degree of vacuolization sometimes approaching the appearance of the wasserhelle hyperplasia, though the largest cells observed are only half the size of those characterizing that condition. In this same island numerous small acini are found with a granular serous secretion in their lumens. In other islands the cells show less than normal vacuolization of the cytoplasm but the nuclei are a little large and distinctly hyperchromatic. A columnar architecture (obscured throughout most of the gland) is rendered prominent by a widening of the vascular channels and an increase in the collagenous stroma. In still another localized area cystic spaces often filled with red cells but without an endothelial lining are seen. This appearance is one we have noted in chief cell adenomas. Occasionally within one of these islands smaller secondary islets can be made out. About the periphery of the larger islands a zone of smaller, more compactly arranged cells is suggestive of compression from the growth of the island itself.

Scattered throughout all the gland singly, in small clusters, and in large nodules, are cells with oxyphil granules in their cytoplasm (Fig. 7). The cells vary in diameter from that of a normal chief cell to three times this size; the granules may be sparsely scattered or so densely packed as to make the cytoplasm apparently homogeneous; there may be extensive vacuolization or there may be none. The nuclei vary from vesicular to pyknotic. In short, the entire gamut of transition stages from chief cell to fully developed oxyphil is present.

A review of the histological features of Case 23A in our former paper, the chief cell hyperplasia with chronic pyelonephritis and osteitis fibrosa, shows that they are in all essentials similar to those of this case. The hyperplasia is diffuse throughout all the glands, the predominant cell is the chief cell; rare acinar formation and occasional wasserhelle cells are observed, whereas transitional and fully developed oxyphils are very numerous. It likewise shows clearly developed islands in which one or another type of cell predominates and around which the surrounding gland tissue seems compressed.

Group 2. Chronic Glomerular Nephritis Without Secondary Bone Involvement

This group is comprised of 12 successive cases of chronic glomerular nephritis in which the parathyroids were available for study.

The pertinent clinical and postmortem findings are summarized in Table I. The duration of symptoms, as far as this can be estimated from the clinical histories, has been given, though a glance at the weights of the kidneys makes it apparent that the renal disease must

TABLE I

Chronic Glomerular Nephritis

No.	Age	Duration	Non-protein nitrogen	Calcium	Phosphorus	Parathyroid			Combined weight of kidneys
						No.	Size	Weight	
	yrs.	*mos.*	*mg./%*	*mg./%*	*mg./%*		*mm.*	*mg.*	*gm.*
1	24	11	200	..	..	2	Normal	..	120
2	30	6	300	..	..	4	8×4×3, 15×8×4 12×5×2, 14×10×4	105, 293 125, 340	75
3	32	8	127	6.35	5.70	4	12×8×5, 3 normal	863	125
4	42	12	180	..	..	4	15×1×4, 8×6×3 2 normal	..	60
5	19	18	205	..	..	4	12×6×3, 9×4×3 10×8×4, 11×8×2	630	100
6	52	12	120	..	..	4	Normal	..	270
7	30	7	190	8.44	8.40 14.42	3	Sl. enlarged	..	150
8	56	30	220	5.91	16.0	4	8×5×4, 3 normal	..	150
9	25	11	105	8.94	5.32	3	1 sl. enlarged 2 normal	..	170
10	26	15	265	8.09	15.65	4	8×5×2, 8×4×3 10×5×2, 7×5×3	360	225
11	26	36	250	7.52	12.13	4	Each 1.2×0.5×0.3	846	120
12	39	16	250	..	..	4	1 sl. enlarged 3 plump	..	240

in most instances have been far more chronic than the symptoms would indicate. They are evidently as a group, however, of distinctly shorter duration than the cases in the preceding group and this undoubtedly accounts for the absence of bone pathology. The vertebral bone marrow was examined in all these cases and found to be negative, except in one which showed slight degrees of bone re-

sorption without, however, any evident fibrosis. The levels of blood calcium and blood phosphate where available are worthy of note. Without exception the calciums are low and the phosphates moderately to markedly elevated, in contrast with the exactly reverse condition — high serum calcium and low serum phosphorus — found in primary hyperparathyroidism.

In this group of cases all but 2 showed gross enlargement of one or more of the glands. In 5 cases enlargement of all the glands was noted (Fig. 10), in 3 cases it was evident in only one, and in 1 case two were enlarged and two were normal. These gross findings are very similar to those of Bergstrand.[7]

With microscopic examination, however, our findings become different. We have found in all of the glands of every case in this group what we consider definite evidence of hyperplasia. Since this hyperplasia is admittedly in some of the cases rather slight, it becomes necessary to examine the criteria on which a diagnosis of hyperplasia can be made.

Let us recall for a moment the architectural features of the normal gland. It possesses a structure rather common among the endocrine glands of anastomosing columns of epithelial cells surrounding vascular spaces which are usually a little wider than ordinary capillaries and approach the character of sinusoids. But in the parathyroids this columnar structure is more complicated than in the other endocrine glands in that it is doubled. Cords 2 to 4 cells wide anastomose about the smaller vascular channels but these are in turn grouped into larger columns 4 to 20 cells in width, which in turn anastomose about the larger vessels and the fibrous stroma of the organ, a stroma, moreover, that is unique among the endocrine glands in that large fat cells are normally present in it in considerable numbers at all times after puberty.

With progressive grades of hyperplasia these fat cells steadily decrease in number and eventually may even disappear. They appear to behave essentially like the fat cells of the bone marrow, modestly giving way to the more important parenchymal cells as need arises. This would explain why some hyperplastic glands are not increased in size, *i.e.* the hyperplasia has progressed only to the point of fat displacement.

The proportion of fat cells to parenchyma, although a very important aid, is by no means an entirely satisfactory criterion since

the "normal" proportion of fat is far from constant, varying significantly with age. Probably the next most useful yardstick is the character of the epithelial columns. Although under normal conditions these show a wide range of variation from 4 to 20 or more cells in thickness, the majority run from 4 to 12 and the thicker ones are found only in limited portions of a gland. With hyperplasia, more and more of the columns are found in the upper ranges and solid sheets of cells without discernible columnar arrangement appear (Fig. 11). Not merely the fat cells but even the fibrous stroma tends to disappear. This widening of the epithelial columns and progressive diminution in the fat and even the fibrous stroma produce a decided compactness of the tissue which is obvious at a glance with low magnification. Though small compact areas may be found in some presumably normal glands, the extension of this appearance to any large proportion of the gland certainly indicates hyperplasia.

Finer cytological details have proved of relatively little help. In the early stages of hyperplasia a tendency to increased vacuolization and simultaneously an increase in the glycogen content, as judged by Best's carmine reaction, is apparent, but with the more marked degrees the cells tend to become smaller once more and most of them revert to the typical chief cell appearance. The search for mitotic figures has, as in the case of the adenomas, proved disappointing. Even in the most extreme hyperplasia — a hundredfold increase in the amount of parathyroid tissue — a 20 minute search with an oil immersion lens has failed to reveal one.

For the sake of clarity the following tabulation of our criteria for the diagnosis of chief cell (secondary) hyperplasia seems worth while.

Criteria for the Diagnosis of Chief Cell Hyperplasia

Gross Appearance:

(*A*) *Size:* Characteristically slight to moderate enlargement of all glands rarely reaching the size of the usual adenoma or primary hyperplasia; however, marked variation is not infrequent in the size of the individual glands of a given case and one or all of them may even be normal in size.

(*B*) *Color:* The glands tend to be a creamy gray rather than an orange-brown.

(*C*) *Consistence:* The glands are firmer and much less pliable than the normal gland, the adenoma or the primary hyperplasia.

Microscopic Appearance:

(*A*) *Low Power*

1. Uniformity of all glands in a given case.
2. Absence or marked decrease in intercellular fat cells.
3. Increase in number of cells as shown by widening of the epithelial columns.
4. Development of compact areas in which columnar arrangement is no longer distinguishable.
5. Tendency to acinar arrangement in the more advanced cases.
6. Uniformity of the whole gland except in the more advanced cases where there is a tendency to adenomatous-like and papillary formations without real encapsulation.

(*B*) *High Power*

1. Cells are ***normal sized*** chief cells, unlike the adenoma or primary hyperplasia.
2. Tendency to vacuolization of the cytoplasm in less severe cases producing slight cell enlargement.
3. No mitoses or hyperchromatism.
4. Oxyphil cells more numerous than expected for age of individual (Fig. 12).
5. Glycogen content slightly higher than adenoma or primary hyperplasia.

Judged on such criteria none of the 12 cases in this group fails to show some evidence of hyperplasia, though in 2 of them it is but slight — not greater than that to be observed in the succeeding group of cases. In the individuals in the present group where gross enlargement was present in one gland only, evident hyperplastic changes have been observed microscopically in the normal sized glands as well and except in degree there has been no difference between the small and the large glands. As compared with the preceding cases with bone involvement, the picture also differs only in degree, the persistence of a few fat cells, and a slightly less degree of island formation and acinar arrangement.

Group 3. Mild Degrees of Secondary Hyperplasia

In a review of the microscopic slides of the parathyroid glands removed from 300 routine autopsy cases (excluding chronic glomerular nephritis), we were able by the use of the criteria listed above, without knowing the anatomical or clinical diagnoses, to select 23 cases in which we believed there was definite hyperplasia. Fourteen of these cases showed, both clinically and pathologically, evidence of some degree of renal insufficiency. Eight of the 14 cases were on the Genito-Urological Service for pyelonephritis; the 9th was a case of congenital polycystic kidneys; the 10th, multiple myeloma with renal involvement; and the last 4 were cases of vascular nephritis. One of the latter had malignant nephrosclerosis of one kidney, the other kidney being atrophic with its pelvis and ureter obstructed by gritty calcified material. The pertinent findings in these cases are given in Table II.

The parathyroid glands removed from the case of polycystic kidneys were surprisingly normal in size. Only two were found and unfortunately they were not weighed. Both of these, however, are alike histologically. There was almost complete disappearance of the intercellular fat cells. Except for occasional single and small groups of oxyphil cells, the glands were composed of chief cells of the transitional wasserhelle type, *i.e.* cells that are on their way to wasserhelle cells. These cells were larger than the normal chief or even oxyphil cell, but did not reach the size of the large wasserhelle cell seen in primary hyperplasia.

One of the 9 cases of pyelonephritis, No. 15 in Table II, showed evidence of very early osteitis fibrosa with characteristic dissecting resorption of the spongiosa, as described by Schmorl [13] and Jaffe.[14] This case might, therefore, fit into the group of renal rickets or our Group 1, but because of the mild degree of bone change, when compared to the other 2 cases, we have preferred not to include it in the renal osteitis fibrosa group.

The remaining 9 cases were not cases of renal insufficiency. Three, however, had bone disease which probably accounts for the secondary parathyroid changes — rickets, metastatic carcinoma and Paget's disease. Whether the latter can really be responsible for the parathyroid hyperplasia is questionable. Two of the cases had duodenal ulcers. One of these, however, showed pituitary basophilism which may account for the parathyroid changes. One of the

TABLE II

Mild Degrees of Secondary Hyperplasia

No.	Age	Duration	Non-protein nitrogen	Phenol-sulphone-phthalein	Calcium	Phosphorus	Parathyroid		Anatomical Diagnoses
							No.	Size	
	yrs.	*yrs.*	*mg./%*	*%*	*mg./%*	*mg./%*		*mm.*	
1	57	¾	36–135	..	..	..	3	2 normal 1 sl. enlarged	Carcinoma of bladder. Pyelonephritis
2	62	5	280	5	..	..	2	1½ normal	Prostatic hyperplasia. Pyelonephritis
3	57	12	81	..	9.79	3.38	4	Normal	Renal stones. Pyelonephritis. Carcinoma of bladder
4	52	31	128	..	..	..	1	Twice normal	Renal stones. Pyelonephritis
5	59	20	..	0–5	9.76	7.90	4	Slight ++	Atrophy of left kidney. Malignant nephrosclerosis of right. Nephrolithiasis
6	49	3	86	Trace	5.2	9.1	2	Normal	Polycystic kidneys
7	35	10	210	5	8.48	11.76	2	1 normal, 6×4×3	Benign vascular nephritis
8	30	6½	88	Trace	..	..	2	1 sl. enlarged. 1 normal	Pyelonephritis
9	58	¼	53	25	9.40	4.96	4	All 8×5×3	Multiple myeloma
10	44	8	66	0–3	..	..	3	Normal	Hydronephrosis. Neurogenic bladder
11	39	?2	220	..	8.81	15.66	3	Twice normal	Malignant vascular nephritis
12	32	10	80	..	7.7	6.4	1	Normal	Benign vascular nephritis
13	54	1½	31	..	..	..	2	Normal	Pyelonephritis
14	27	3½	20	25	..	..	1	Normal	Nephrolithiasis. Pyonephrosis
15	17	3	150	5	..	..	4	Twice normal	Congenital anomalies of bladder and ureters

remaining 4 cases had malignant hypertension with slight chronic vascular nephritis, but with a non-protein nitrogen of only 24 and a phenolsulphonephthalein of 40 per cent, it was felt that this case did not belong with the group of renal insufficiency cases. The remaining 3 cases — bronchial asthma, rheumatic heart disease, and metastatic carcinoma of the lung from a carcinoma of the cecum — showed nothing in the kidneys that could be responsible for the parathyroid changes. Table III gives abstracts of the cases in this group.

TABLE III

Hyperplasia in Non-Renal Cases

No.	Age	Parathyroid		Anatomical Diagnoses
		No.	Size	
1	*yrs.* 12	1 (operation)	Normal *mm.*	Rickets
2	36	..	...	Oat cell carcinoma of lung with metastases to bones
3	64	4	Normal	Paget's disease of bone. Glioma
4	67	3	2 normal. 1 (8×5×4—80 mg.)	Duodenal ulcer, gastritis, pituitary basophilism
5	55	3	Normal	Duodenal ulcers for 25 yrs. Had taken soda bicarbonate for yrs.
6	42	3	Normal	Hypertension. Chronic vascular nephritis, slight
7	65	1	Normal	Rheumatic heart disease
8	64	3	Normal	Bronchial asthma
9	33	4	Normal	Metastatic carcinoma of lung from cecum

On gross examination most of the glands in this group showed very slight but definite enlargement. The microscopic picture, however, was similar to but not so pronounced as most of those in the group of chronic nephritics. Here, intercellular fat cells were more numerous in some of the glands, but others showed a definite compactness of structure, vacuolization of cells and pseudoglandular arrangement.

Discussion

The exact mechanism of the parathyroid hyperplasia in chronic renal insufficiency is still a subject of active debate and no attempt will be made to discuss it here. Phosphate retention, however, is generally admitted to be the initial stimulus and this concept has recently been supported by the production of parathyroid hyperplasia in rabbits by Drake, Albright, and Castleman [15] by the repeated injection of a neutral buffered isotonic solution of sodium phosphate. The hyperplasia so produced is essentially similar to that described in this paper, being characterized by a great increase in the number of chief cells, chief cells, moreover, of normal size and appearance. The only significant difference lies in the lack of oxyphils in the experimental hyperplasias. This may depend on a species difference or may merely be due to the fact that the experiments were not of sufficiently long duration.

Our cases have been presented in four groups: (1) marked chronic renal insufficiency associated with osteitis fibrosa; (2) chronic glomerular nephritis; (3) milder degrees of renal insufficiency; and (4) a group of 9 cases showing parathyroid hyperplasia but without any degree of renal insufficiency. In the first group in which the hyperplasia was most marked and in which bone lesions were also present we have reliable clinical data pointing to a state of severe renal insufficiency of many years duration. In the next group, chronic glomerular nephritis, the duration of the renal insufficiency as judged by the clinical history and by the degree of renal atrophy was also marked but not so great as in the first group. Correspondingly the degree of parathyroid hyperplasia was not so great. The third group presented, showing minor grades of hyperplasia, was made up of a variety of types of renal pathology including nephrosclerosis, polycystic kidneys and pyelonephritis, for the most part secondary to other pathological conditions in the male genito-urinary tract. Although nephrosclerosis and polycystic kidneys represent very long-standing renal disease, functional renal insufficiency is apt to appear only in the terminal stages and to be of comparatively short duration. The fact that all of our cases of chronic glomerular nephritis showed parathyroid hyperplasia appears at first hand quite at variance with Bergstrand [7] who found hyperplasia in only 20 per cent of his nephritics. His paper, however, includes no clinical data and, so far as the negative group is concerned, no classification as regards

types and severity of nephritis from which an estimate of the degree and duration of renal insufficiency can be made.

The studies which we have reported in our previous paper combined with these just presented indicate that histological examination of the parathyroid glands will ordinarily readily permit the distinction between primary and secondary hyperparathyroidism. Primary hyperparathyroidism is the result either of a tumor-like enlargement of one or part of one gland, or of a diffuse hyperplasia of all the glands, sharply characterized by the uniform wasserhelle character of all the cells. Secondary hyperplasia, in contrast, though likewise showing as a rule uniform and sometimes marked enlargement of all the glands, fails to show the same orientation of all the cells toward one line of differentiation. Though chief cells greatly predominate, wasserhelle cells, although much smaller than those seen in primary hyperplasia, are by no means totally suppressed and oxyphil cells are regularly greatly increased in number. With adequate data confusion between these two types of hyperplasia is scarcely possible. However, in view of the inadequate data of many of the cases in the literature, and before we realized the difference in histology and clinical findings, we undoubtedly erred in interpreting some of them.

In our previous paper in the classification of the cases reported in the literature we listed 14 cases of wasserhelle cell hyperplasia and only 2 cases of chief cell hyperplasia. We also listed 10 cases of multiple adenomas of the parathyroid. A review of these 26 cases in light of our further knowledge on the subject calls for a definite reclassification. Probably not more than 5 out of the 14 listed cases of primary hyperplasia really belong to this group. Many of the others belong to the secondary chief cell type, but the majority cannot be accurately classified because of insufficient data. This same criticism might be applied also to some of the 11 cases cited from the literature in the paper by Albright, Bloomberg, Castleman and Churchill [16] in the first report of primary hyperplasia.

A review of the literature, however, suggests the possibility of confusion with the primary adenomas since enlargement of a single gland in association with nephritis has been described on several occasions. Most of these reports are based solely on gross examinations and some of Bergstrand's findings agree with ours — that significant grades of hyperplasia may be present without enlarge-

ment of the gland. It seems fair to assume that in many of these cases histological examination would have revealed significant changes in the other glands.

That a primary parathyroid adenoma should occasionally be found in a patient suffering from chronic nephritis is by no means impossible and it seems probable that MacCallum's [6] case is to be explained in this way. Dr. Fuller Albright has reviewed this case with Dr. MacCallum. They found true osteitis fibrosa and felt that the case was one of a true parathyroid adenoma.[17] Seven of Bergstrand's 10 cases evidently correspond to ours in all essentials. In the remaining 3 enlargement was limited to one gland; the remaining glands were examined histologically and were considered normal. In 1 of these cases, moreover, the abnormality was limited to an adenomatous growth in only a part of the gland. Any doctrinaire opinion of these findings is obviously unwarranted. It is possible that minimal grades of diffuse hyperplasia were overlooked but it is also remotely possible that localized, adenoma-like hyperplasia is occasionally the response of the parathyroid glands in secondary hyperplasia. A tendency in this direction — toward the development of localized, apparently semi-autonomous centers of excessive growth or of peculiarities of differentiation — has been described in our 2 most advanced cases. However, in these instances obvious hyperplasia was evident in the remainder of the gland and also in each of the other glands from the same case, and, moreover, in all of the 38 cases included in this report, not merely those associated with renal insufficiency, hyperplasia when present in one gland was also evident in all the other glands from the same patient. We are, therefore, prejudiced against the occurrence of localized hyperplasia. The study of a much larger amount of material will evidently be necessary to reach a conclusion on this point.

The cause of the gross enlargement of the parathyroid tissue in the various types of hyperparathyroidism has aroused some conflict of opinion. In primary hyperparathyroidism of both the adenomatous and the hyperplastic types a tendency to enlargement of individual cells is very evident. Some authors have felt that this macrocytosis alone without an increase in the number of cells was adequate to explain any grade of enlargement they had seen. As far as the adenomas are concerned it certainly would be difficult to explain a tumor such as that in Case 1 of the Massachusetts General

Hospital series. This tumor weighed 53.2 gm. and although occasional large cells (30 μ) are present, the average cell is not over 15 μ in diameter. Macrocytosis may explain a tenfold increase in size but hardly a thousandfold one. Another element tending to increase the gross mass of the glands is the presence in them of dilated acini and cysts containing fluid. In some of the adenomas and most of the primary hyperplasias this must be a significant factor in the weight of the glands. Quantitative studies of the influence of these two factors in relation to the adenomas and especially to the primary hyperplasias are in progress and any opinion would be hazardous without such data.

In the secondary hyperplasias, however, there is no room for argument. The degree of glandular enlargement may run close to a hundredfold, as in 1 of the cases presented, but the predominant cell is a normal sized chief cell and, though larger water-clear and oxyphil cells are present, they are not numerous enough to affect the size of the glands significantly. Moreover, acinar formation is limited and acini where present show small lumens without significant accumulation of fluid. Yet even in these cases where an extraordinary multiplication of cells must have taken place mitotic figures have not been observed.

In our former paper an attempt was made to infer from the cytological evidence presented the probable interrelations and functional significance of the three main types of cells that make up the normal parathyroid. Does the study of secondary hyperplasias carry us any farther in this direction? As regards the interrelation of the cells, it is strongly confirmative of the monophyletic theory. In the normal glands and in the adenomas the fundamental cell was found to be the chief cell and all stages of transitional steps between it and the water-clear cells and the oxyphils were noted. In normal glands such transitional cells must frequently be searched for, but in the active, highly cellular secondary hyperplasia they are extremely numerous, particularly highly vacuolated cells with considerable dense acidophilic cytoplasm which show characteristics of both the water-clear and the oxyphil cells.

The findings in this group of cases appear to fit the hypothesis of a slow progression of development from the chief cell, through the water-clear to the oxyphil. The possibility of direct development of oxyphils from the chief cells cannot be excluded however. We

would suggest that in secondary hyperplasia the rate of this progression is increased, thus accounting for the much greater number of oxyphils than would be expected for the age groups of the patients. The presence of large numbers of oxyphils would on this basis interfere in no way with the theory previously advanced — that they are essentially functionless — since the greatly predominant chief cells would account for the presumable increase in function.

Summary and Conclusions

Another case of "primary" hyperparathyroidism characterized by diffuse hyperplasia of the parathyroid glands of the wasserhelle type is reported. The histological findings in this case have been used to emphasize the contrasting character of the "secondary" hyperplasia which is described in detail on the basis of 29 cases of chronic renal insufficiency of varying grades. Whereas in the primary hyperplasias a uniform direction of differentiation of all cells to the large water-clear type is the invariable finding, in the secondary hyperplasias such uniformity is lacking. Here the glands are composed almost completely of normal sized chief cells, although a few small water-clear cells are occasionally present. The oxyphil cells are always greatly increased in number. The glands show varying degrees of gross enlargement and even when the enlargement is limited to a single gland, microscopic examination has not failed in any instance to show evident hyperplasia in the other glands as well. The criteria for the diagnosis of secondary hyperplasia are described. Comparison of cases of chronic renal insufficiency with and without bone lesions showed quantitative but not qualitative differences in the parathyroid glands, and the development of osteitis fibrosa is felt to be directly dependent on the duration of renal insufficiency. That these changes are in no way specific to renal insufficiency is shown by the fact that no qualitative differences could be recognized between the milder grades of secondary hyperplasia in nephritis and those occasionally seen in individuals without renal insufficiency, but with a variety of associated lesions varying from metastatic carcinomatosis involving bone to basophilism of the pituitary.

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DESCRIPTION OF PLATES

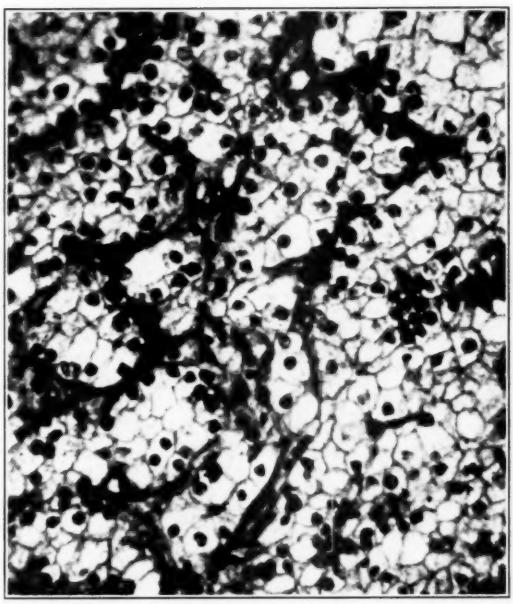
PLATE 89

FIG. 1. A diagrammatic drawing of the parathyroid glands *in situ* from a case of primary hyperplasia. Note the pseudopod-like projection in the upper glands.

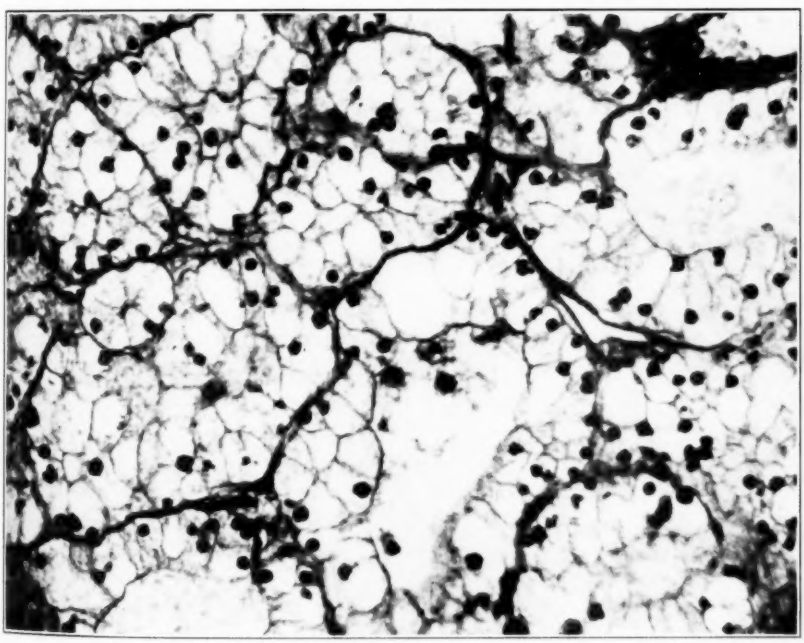
FIG. 2. A microphotograph of a section of one of the glands in Figure 1 showing the basal orientation of the nuclei producing a characteristic pattern. × 500.

FIG. 3. A microphotograph of a section of one of the glands from the same case and at the same magnification. The large wasserhelle cells show a definite tendency to glandular arrangement. Note the resemblance to the hypernephroma cell. × 500.

1



2



3

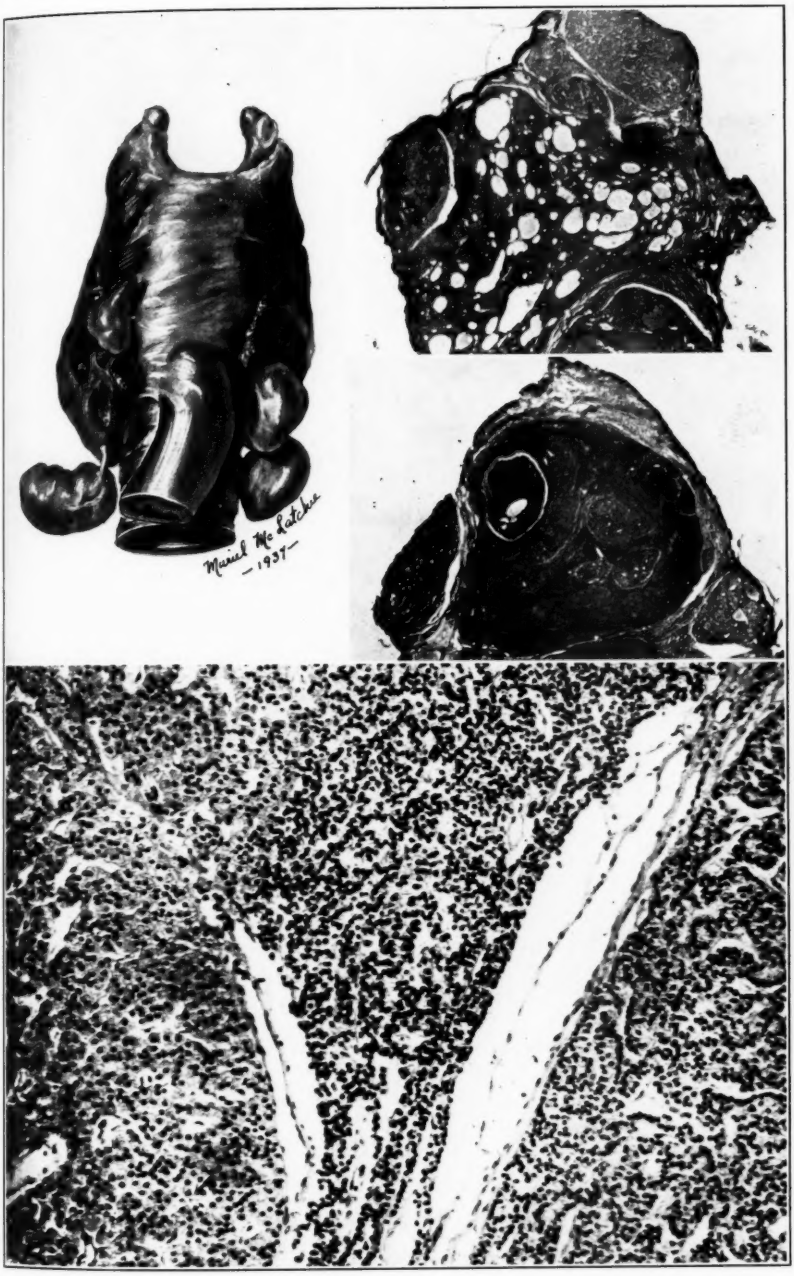
Castleman and Mallory Parathyroid Hyperplasia in Renal Insufficiency

PLATE 90

FIG. 4. A drawing of the parathyroid glands in a case of parathyroid hyperplasia secondary to long-standing chronic renal disease.

FIGS. 5 and 6. A low power microphotograph of a section of one of the glands in Figure 4 showing the circumscribed encapsulated islands of cells. × 10.

FIG. 7. A higher power of several of the islands in Figure 5 showing the variation in the type and arrangement of the cells in the different islands. Note the islands of oxyphil cells on the left. × 150.



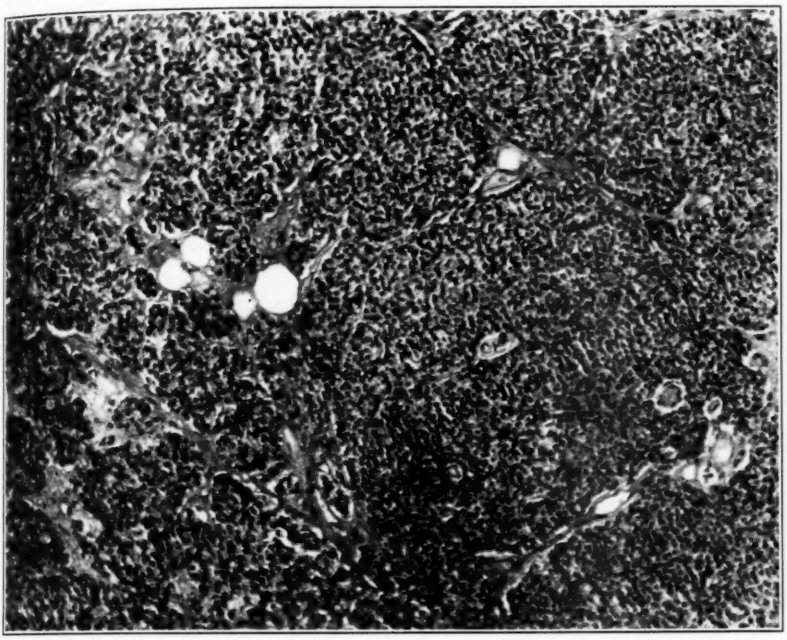
Castleman and Mallory

Parathyroid Hyperplasia in Renal Insufficiency

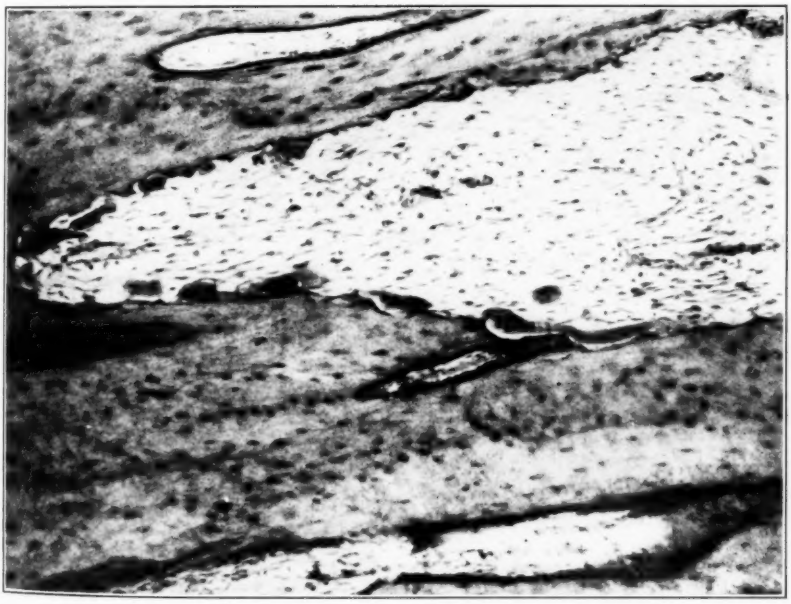
PLATE 91

FIG. 8. A microphotograph of a section of one of the glands seen in Figure 4. Note the cellular compactness, almost complete absence of fat, and the normal sized chief cells. In this gland the nodularity and island formation is relatively inconspicuous. × 150.

FIG. 9. A microphotograph of a section of vertebra from the same case showing well marked osteitis fibrosa. Note the large numbers of osteoclasts at the edge of the bone trabecula. × 500.



8



9

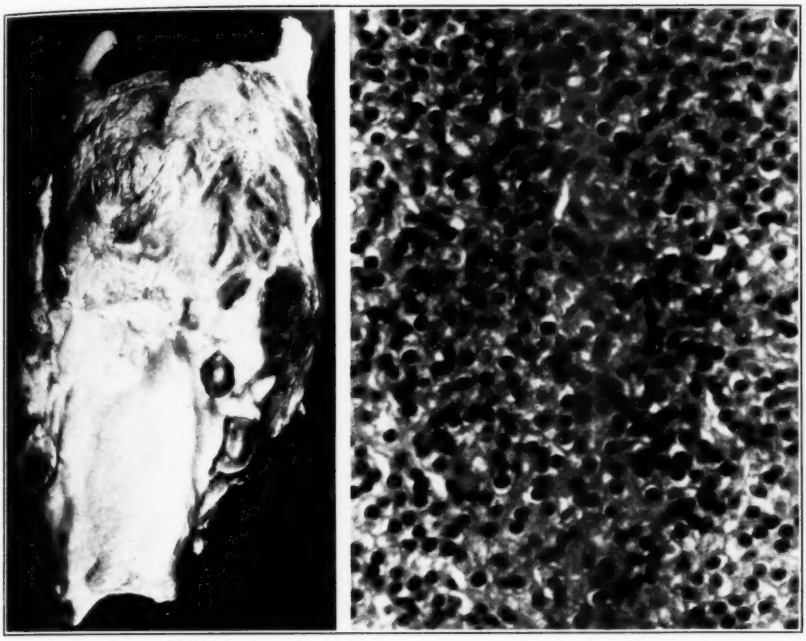
Castleman and Mallory Parathyroid Hyperplasia in Renal Insufficiency

PLATE 92

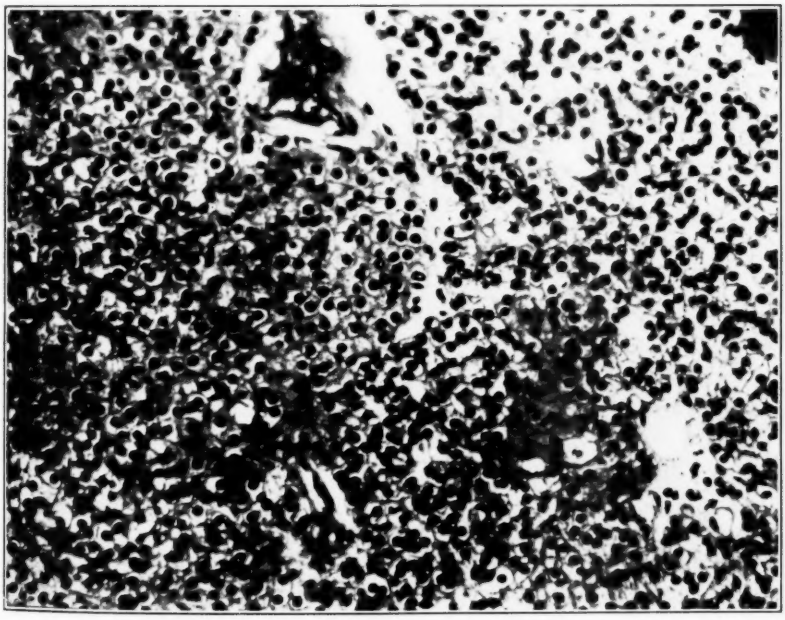
FIG. 10. A photograph of the parathyroid glands in a case of chronic glomerular nephritis. Case No. 10 in Table 1. Note the enlargement and plumpness of the glands.

FIG. 11. A microphotograph of a section of one of the parathyroid glands in a case of chronic glomerular nephritis showing the solid sheets of cells without discernible columnar arrangement. × 500.

FIG. 12. A microphotograph of a section of one of the parathyroid glands in another case of chronic glomerular nephritis showing the slight vacuolization of the cytoplasm in some areas and the increased number of oxyphil cells. × 500.



10 11



12

Castleman and Mallory Parathyroid Hyperplasia in Renal Insufficiency

THE RESPONSE OF GUINEA PIG BONE MARROW TO LIVER EXTRACT *

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Interest in hemopoiesis has received considerable impetus in recent years as the result of the morphological studies of Sabin *et al.*,[26, 27, 28] Doan *et al.*,[6, 8] Jordan and Johnson,[15] Muller,[20] and Peabody.[23, 24] It is generally accepted that very little comprehension of peripheral blood changes obtains without understanding of the basic structural factors underlying these phenomena. Such factors must be sought fundamentally in the histological structure of the bone marrow.

Recently it has been shown by Jacobson [11, 12] that "normal" adult male guinea pigs fed a diet of oats, carrots and lettuce react to the administration of liver extract by the development of a small but significant increase in the number of circulating reticulocytes. Such a response, if constant and consistent, would permit the utilization of this animal as a means of biological assay of the potency of the various liver fractions now in the process of isolation.[16, 18, 31] Hitherto, the only satisfactory means of testing these substances has been their administration to individuals in the relapsed phase of pernicious anemia. The value of utilizing guinea pigs for this procedure is readily appreciated, particularly in view of the absence of any reticulocyte increase, either spontaneously or following the administration of impotent substances.[13] Unfortunately, however, other investigators have questioned the reliability of this test in view of the fact that in their hands guinea pigs showed reticulocyte responses following the administration of materials known to be impotent in pernicious anemia.[1, 10] Definite reticulocyte crises also were said to occur spontaneously.

In this publication the marrow of the guinea pig has been studied under several experimental conditions in order to determine the effect of liver extract and to seek, if possible, the explanation of the disagreement among contemporary investigators. A control series was adequately obtained in a group of stock guinea pigs and no

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attempt was made to utilize other potential reticulocytogenic materials in addition to the liver extract, despite the fact that many such substances are known to exist.[19, 20]

Experimental

Five groups of guinea pigs were studied. All were composed of adult male animals weighing more than 500 gm. No other general restriction was imposed except for those noted below.

Group I consisted of 6 pigs obtained from the stock used by Jacobson. These animals had been kept under the conditions outlined by him [12] and had subsisted on the prescribed diet for a period of 1 month or longer. They had never received liver extract but were known to have stable peripheral reticulocyte levels.

Group II consisted of 4 pigs obtained from the same stock. These, however, had received liver extract in the past and were known to be consistent, satisfactory reactors. They had received no liver for a month or longer preceding the experiment.

Group III consisted of 7 pigs obtained from the same stock. These animals were also known reactors. They were given by intraperitoneal injection a single test dose of liver extract (commercial extract derived from 4.3 mg. of whole liver per kilogram of pig weight) and daily reticulocyte counts were performed. All 7 showed the expected reticulocyte response (Table I) and were killed on the day following that on which the reticulocyte peak was reached.

Group IV consisted of only 2 pigs of the same stock and was merely included to supplement Group III. An attempt was made here to determine whether or not repeated daily test doses of liver extract (extract derived from 4.3 mg. of whole liver per kilogram of pig weight) would produce an accumulative effect on the bone marrow. Repeated doses were given for 6 and 10 days respectively and the animals were killed at the first indication of subsidence of the reticulocyte response (Table II). Occasionally daily red blood counts were omitted in order to avoid marrow changes resultant on the repeated loss of small amounts of blood.

Group V consisted of 20 pigs obtained from the general laboratory stock. These had not been sustained under the conditions outlined above but had subsisted on a diet of hay, oats and carrots under such conditions as obtained with the modicum of attention and care usually directed toward these animals. Almost all of the animals had

received an injection of suspected tuberculous material 2½ to 3½ months preceding the experiment. They were autopsied and examined meticulously for any evidence of disease and all included in this group were normal in appearance. Since all material of the type noted was routinely injected into pairs of guinea pigs, a double check

TABLE I

Group III. Reticulocyte Response to Liver Extract

Guinea pig No.	Liver extract injected	Date of reticulocyte count	Reticulocyte count
			%
509	4/6/36	4/6/36	0.8
		4/7/36	0.8
		4/8/36	1.6
		4/9/36	2.4
		4/10/36	3.0
		4/11/36	2.2
511	4/6/36	4/6/36	1.0
		4/7/36	1.2
		4/8/36	1.4
		4/9/36	2.2
		4/10/36	2.8
		4/11/36	2.4
523	4/6/36	4/6/36	1.3
		4/7/36	1.0
		4/8/36	1.8
		4/9/36	2.0
		4/10/36	2.8
		4/11/36	2.8
503	5/4/36	5/4/36	1.2
		5/5/36	2.2
		5/6/36	3.2
		5/7/36	3.0
510	5/4/36	5/4/36	1.2
		5/5/36	2.4
		5/6/36	3.4
		5/7/36	3.0
508	5/4/36	5/4/36	1.0
		5/5/36	2.0
		5/6/36	3.2
		5/7/36	2.8
512	5/4/36	5/4/36	1.0
		5/5/36	2.0
		5/6/36	3.0
		5/7/36	3.0

was obtained. No pig was used the partner of which showed evidence of disease. The length of time intervening between the injection and sacrifice was considered sufficiently long to obviate attributing any effect to the substance injected.

All of the animals were killed by a sharp blow on the head. Both femora of each pig were immediately disarticulated and dissected

TABLE II

Group IV. Peripheral Blood Response to Repeated Doses of Liver Extract

Guinea pig No.	Date	Liver extract injected	Reticulocyte count	Red blood count
			%	*million*
561	6/29		1.0	
	6/30		1.4	
	7/1		1.4	
	7/2		0.4	
	7/3		1.0	
	7/4	7/4	0.8	
	7/5	7/5	0.8	
	7/6	7/6	1.4	
	7/7	7/7	1.8	
	7/8	7/8	2.2	
	7/9	7/9	2.4	
	7/10		1.8	
555	7/27	7/27	1.2	5.04
	7/28	7/28	1.0	4.54
	7/29	7/29	1.6	4.78
	7/30	7/30	1.8	4.54
	7/31	7/31	1.8	4.52
	8/1	8/1	2.4	—
	8/2	8/2	3.0	5.6
	8/3	8/3	3.0	4.85
	8/4	8/4	4.0	—
	8/5	8/5	2.8	5.76
	8/6		2.0	5.73

free of the attached musculature. The bone was then split and the readily detached pencil of soft, gelatinous marrow was removed. In many of the animals the marrow of the tibia and humerus was also removed and treated in a similar manner. The sternum was occasionally removed, split and immediately placed in a fixative with the marrow intact.

Each pencil of marrow was placed on a dry paper towel and arranged in a concentric spiral fashion so that after fixation a cut section included all levels of the marrow. In many of the specimens

2 minute drops were removed by gentle suction with a capillary pipette. These were transferred to slides previously prepared for supravital staining with a dried film of neutral red and Janus green and studied in the fresh state.[28] As a routine measure, however, imprint preparations were made of all specimens and these were stained by a combination of Wright's and Giemsa's stains.

The marrow was allowed to remain attached to the paper and this was trimmed close to the specimen, after which both marrow and the small segment of paper were dropped into Zenker-formol. The tissue was treated according to the method outlined by Custer,[3] embedded in paraffin, and sectioned as thin as possible. Our preparations averaged about 5 to 6 μ in thickness.

The supravitally stained preparations were used purely for confirmatory histological purposes. Attempts to use them for absolute differential counts were in many cases unsuccessful because of the dense cellularity encountered. Counts of 1000 to 2000 cells were, however, performed on each of the Wright-Giemsa stained preparations. In these, excellent cellular differentiation was obtained within the thinned out periphery of each imprint, but because of irregular distribution the impossibility of identifying the elements in the thick central portion of the imprint, and the imperfect correlation with the evident proportions in the sections, no attempt is made to stress the findings in the present paper.

The observations served, however, to familiarize the author with certain morphological characteristics of the guinea pig marrow constituents and they were used only in a corroborative sense in drawing conclusions.

Critical attention was directed primarily toward the fixed sections and these offered the opportunity for several important observations. Each section was meticulously examined for relative cellularity and the general distribution and arrangement of its components. The changes observed form the basis for this publication. Relatively little difficulty was encountered in identifying the great majority of the constituent elements but the variation in distribution (Fig. 3), the great tendency toward grouping of the less mature elements, and the importance of the mature erythrocytes, accurate counts of which were impossible, precluded the determination of absolute percentage proportions. Additional and applicable data were obtained, however, in the following manner. Each section was ex-

amined with the low power lens to determine general cellularity, fat infiltration, and the presence of bone spicules. With the high dry objective 20 or more different fields were examined in representative portions of the marrow with the purpose of determining again relative cellularity, the number, size and variability of fat vacuoles, and the distribution of megakaryocytes. Oil immersion magnification was utilized to determine the relative proportions and arrangement of immature cells of all types and the same characteristics of eosinophiles, normoblasts, neutrophiles and mature erythrocytes.

It is believed that analyses of these elements suffice to establish subsequent conclusions but brief attention will first be directed toward a single element that has occasioned some disputing comment, *i.e.* the megaloblast. Jacobson [11, 12] stated that the guinea pig marrow was megaloblastic in character and that the reticulocyte responses to the administration of liver were probably attributable to this fact. Jones,[14] on the other hand, claimed that the elements named by the former author were not megaloblasts but proerythroblasts. It is not my intention to intrude on a long-standing controversial point, but certain facts are quite evident. The marrow of none of the pigs examined was megaloblastic to the extent that such a term implies. There were identified in all of the marrow imprints and supravital preparations, however, cells that have been considered to be megaloblasts (Fig. 4). Similar cells have been regularly observed in fetal liver and pernicious anemia bone marrow imprints but have never been noted personally in normal human bone marrow or in that obtained from individuals suffering from the various types of hypochromic anemia. Tötterman [32] has recently observed these cells in the bone marrow of a patient suffering from hemolytic icterus.

In the Wright-Giemsa preparation the cell measured 14 to 20 μ in diameter and contained a round or oval shaped, centrally placed nucleus with a scanty rim of deeply basophilic homogeneous cytoplasm. The chromatin was arranged in a fine, fairly regular, shreddy reticular network with a definite parachromatin often exhibiting a vague scroll-like appearance similar to that described by Dameshek.[4] Such a cell answers the description of a megaloblast given by Ferrata [9] and Naegeli.[21] *

The appearance of the cell contrasts definitely with that of the

* The identity of this cell as a megaloblast has been confirmed by Dr. Florence R. Sabin in our imprint preparations.

myeloblast (Fig. 4) which possesses a closely packed stippled chromatin with poorly distinguishable parachromatin. Nucleoli are almost always present in the latter cell and vary from 1 to 5 in number. In the megaloblast, however, there are usually none, although occasionally 1 or 2 are seen. Transition stages between this cell and early erythroblasts are often noted. These manifest themselves by a greenish tint to the partially hemoglobinized cytoplasm, an increase of the thickness and diminution of the shreddiness of the chromatin, and an increased prominence and sharpness of outline of the parachromatin.

In the supravital spreads the cell measures between 15 and 25 μ in diameter and retains the nuclear-cytoplasmic ratio noted above. The cytoplasm has a hazy homogeneous yellowish tint indistinguishable from that noted in the immature cells of the myeloid and lymphoid series but considerably less in intensity than that noted in the fully hemoglobinized late erythroblasts. The color is probably not resultant on any hemoglobin content. A scanty circlet of medium sized coccoid mitochondria usually adheres closely to the nuclear margin and no neutral red staining elements are present.

Whatever this cell may be termed it was present to the extent of 0.1 to 3.5 per cent of the nucleated elements in all of the imprint preparations. A greater prominence is noted in Groups I, II and V. In Group V the values range from 1 to 3.5 per cent, the majority containing more than 2 per cent. In Groups I and II the values range from 0.7 to 1.4 per cent and in Groups III and IV between 1.1 and 0.6 per cent. The significance of these variations will be discussed below.

Examination of the sections revealed several other phenomena that differed from normal human marrow. The increased cellularity of the marrow of the long bones was, of course, one such point. Furthermore, there were relatively enormous numbers of eosinophiles and in certain of the groups megakaryocytes were remarkably abundant. Lymphocytes were consistently present in numbers approaching 15 per cent of the nucleated cells but at no time were follicles observed. In deriving the data noted below, however, the possibility of criticism directed toward inexact morphological distinction in so modified a medium as a fixed and paraffin infiltrated section was considered and only such elements as would permit minimal individual misinterpretation were included.

Group I: (Animals fed oats, carrots and lettuce for a period of 1 month or longer without ever having received liver extract.) All of these guinea pigs showed densely cellular marrows with relatively few fat vacuoles and very few bone spicules or areas of fibrosis (Fig. 1). Only two of the specimens showed occasional, small, less cellular areas with increased numbers of vacuoles. In these areas, however, the sinusoids were dilated and contained large numbers of nucleated cells. For the most part the fat was widely scattered and most of the vacuoles were small. They averaged 5 per high power field. All of the sections showed a large number of immature cells, the great majority of which were of the myeloid series. There were many foci in which these cells were sufficiently numerous to constitute the predominant cell in these regions. Mitotic figures were present in remarkably small numbers. Many of the sections showed large numbers of eosinophiles which were present to the number of 35 in the average oil immersion field. Neutrophilic leukocytes were present in large but varying numbers. The less mature band forms were twice as numerous as those containing segmented nuclei. Myelocytes and other immature myeloid cells were present in about an equal proportion in all 5 groups of pigs, but they were more numerous in relation to the mature myeloid cells in only this Group and in Group II. Megakaryocytes were numerous and immature forms of this type of cell were equally as frequent as the mature forms. Occasional high power fields contained as many as 15 of these but the average field in all 6 pigs contained 6. Moderate numbers of normoblasts were diffusely scattered throughout the section but only rarely was any suggestion of island formation discerned. Mature red blood cells were scanty, scattered sparsely, and only occasionally was there any evidence of grouping. So few red blood cells were evident in the sections that only with pointed search were more than occasional erythrocytes noted (Fig. 1*a*).

Group II: (These pigs received the same diet as those described in Group I but had received liver extract in the past and were known to be consistent reactors. They had received no liver for a month or longer preceding the experiment.) The animals exhibited marrows that were indistinguishable from those in Group I. There was dense cellularity and a very small number of vacuoles. Megakaryocytes were abundant and mature red blood cells sparse. Immature cells of the erythroid and myeloid series were slightly increased and

polymorphonuclear cells were abundant. Here also band forms were much more numerous than the segmented. Moderate numbers of normoblasts were diffusely scattered and there was remarkably little grouping of these elements (Fig. 1*a*). Eosinophiles were conspicuously numerous.

Group III: (Animals similar to those in Group II which were sacrificed at the peak of a reticulocyte response to a single test dose of liver extract.) The marrows of these pigs exhibited striking differences from those of the preceding 2 groups. There was moderate variation in some of the sections but on the whole each section showed a definite dimunition of cellularity. Vacuoles were enormously increased in size and number and averaged 35 per high power field (Fig. 2). In many areas only scantily filled sinusoids interposed between adjacent fat globules and would have suggested hypoplasia were it not for concomitant changes. Sinusoids barely discernible in Groups I and II were quite obvious in this group. Intersinusoidal capillaries described by Doan,[5] apparently unconnected with sinusoids, contained varying numbers of mature and immature cells. In general the nucleated cellular content of the sinusoids was definitely diminished and the numbers of mature red blood cells considerably increased (Fig. 2*a*). Even in persistently cellular areas erythrocytes were clearly evident. They were distributed in a diffusely scattered fashion and also appeared as solid cords, as though lying within sinusoids indistinguishable because of the adjacent cellularity. In many areas these erythrocytic cords became widely dilated to form lakes, a feature that was even more noticeable in Groups IV and V. Scattered normoblasts were present in unchanged numbers per unit field but were more manifest as the result of the generally diminished compactness of architecture. Many fields, however, contained large islands of normoblasts aggregated in such a fashion that they were prominently evident, even when viewed with low power objectives (Figs. 2 and 2*a*), a feature lacking in the initial two groups. Less mature erythroid elements were considerably diminished. Myelocytes and earlier myeloid cells were likewise decreased and were limited to a few, scattered shrunken islands. Mitotic figures were scanty. Megakaryocytes were fewer and were present to the average extent of 1 per high power field. No field contained more than 4 and mature forms of this type of cell predominated. Eosinophiles were likewise less prominent

and numbered about 19 per oil immersion field. Polymorphonuclear leukocytes were slightly diminished but this decrease was relatively greater among the band forms and the ratio of segmented to non-segmented nuclei was only 1:1. More fine bone spicules were present than in Groups I and II, and there was also a slight degree of scattered fibrosis.

Group IV: (Given daily test doses of liver extract for 6 and 10 days respectively.) The 2 pigs in this group exhibited some resemblances to all of the preceding 3 groups. There was relatively dense but varied cellularity with only 12 various sized fat vacuoles within the average high power field. Distinguishable sinusoids were distended and filled with cells. These cells, however, were for the most part non-nucleated red blood cells. Throughout the entire section the erythrocytes and normoblasts were numerous, the latter occurring frequently in large islands. Immature cells of both series were likewise present in fairly large groups and mitotic figures were frequent. Eosinophiles were diminished to 16 per oil immersion field and neutrophiles were much increased in numbers. This latter group showed a ratio of segmented to non-segmented forms of less than 1. Megakaryocytes were present in as large numbers as were noted in Groups I and II but all of these were mature in appearance. There were also in the pig killed at the end of 6 days many multinucleated giant cells with vacuolated cytoplasm. The characteristics of these marrows were rather puzzling and for a brief period they remained so until analyses of the next group were completed.

Group V: (Animals obtained from the general laboratory stock fed a diet of hay, oats and carrots.) Here, as in the preceding group, there were resemblances to Groups I, II and III. Its distinction in this respect suggested a close relation to Group IV, a presumption that was borne out by the data obtained. The sections showed considerable variability of compactness (Fig. 3). Immediately adjacent to a densely hyperplastic area there often lay one practically devoid of hemopoietic tissue wherein there were large numbers of fat vacuoles. The intervening sinusoids were narrow, compressed and relatively acellular. Such areas occurred as frequently within the diaphysis as they did in the metaphyses, and additional sections cut from deeper levels in the block showed persistence of this arrangement. In general, however, the cellular areas predominated. Bone spicules varied considerably in number and there were rare scattered

foci of fibrosis. Sinusoids were both acellular and cellular in appearance, the latter being packed with nucleated and non-nucleated elements. Immature myeloid and to a lesser degree erythroid cells appeared in large groups, occasionally intermixed, and mitotic figures were focally abundant. Megakaryocytes were present in numbers equal to those in Groups I and II and showed no diminution even in extensively vacuolated regions. Less mature members of this group were again significant. Eosinophiles were abundant throughout and often exhibited narrow strands extending through compressed sinusoids, the sole occupants of these spaces. There were many diffusely distributed polymorphonuclear cells which occasionally encircled compact islands of immature precursors. In this series, again, less mature cells predominated, the ratio of non-segmented to segmented forms being 4:1. Normoblasts were particularly numerous and demonstrated extensive grouping (Fig. 3*a*). Frequently in hyperplastic segments they appeared as loosely arranged halos about islands of erythroblasts. The non-nucleated red blood cell content was somewhat greater than that of Group III but approximately equivalent to that of Group IV. Here, however, many narrow solid strands of erythrocytes were observed coursing through the denser areas and a few lakes were encountered. Table III demonstrates the contrast of characteristic features in the bone marrows of the various groups.

Discussion

The initial purpose of this paper was to determine whether the guinea pig showing a peripheral blood response to liver extract would exhibit also a constant change in the bone marrow. It was hoped, too, that the significance of this change, if present, would be established and that in so doing an explanation would be offered for the variance in the results obtained by the different investigators.

Examination of the marrows of Groups I and II, which obviously were entirely similar, demonstrated fundamentally an intensive cellularity with a preponderance of relatively immature forms (Fig. 1*a*). Early erythroid and myeloid cells were quite prominent and exhibited themselves in large, poorly circumscribed masses. Megakaryocytes, particularly in immature forms, and eosinophilic leukocytes were very numerous. The striking feature, however, was the relative dearth of mature erythrocytes and the absence of well

defined foci wherein evidence of red blood cell maturation was forthcoming. True, normoblasts were present in fairly significant numbers but the numerous large islands visible in the other 3 groups were extremely scanty here.

In view of these and the other observations noted above it is readily conceivable that such marrows have received minimal developmental stimulation resultant presumably on some dietary deficiency. Actual generative powers are unimpaired and the potentiality of immediate maturation or acceleration of preexisting adequate but minimal blood development is obviously the prime characteristic of this marrow.[17] Certainly, the striking changes evinced in Group III demonstrate convincingly that liver extract contains a factor deficient or lacking in the first 2 groups and, further, that its administration produces a definite effect on the histological appearance of the hemopoietic system. Since no other factor entered into the experiment, except for the administration of this substance, such a conclusion is warranted. The guinea pigs of Groups I, II, III and IV were from the same animal room, kept under exactly similar conditions, and were chosen at random from this stock for the several procedures. The only stipulation observed, as has been previously noted, was that those pigs in Groups II, III and IV should be known consistent reactors to liver extract. Seasonal variations noted by Starkenstein [29] were excluded by reason of the fact that animals of all 5 groups were killed at irregular intervals within brief periods of one another. There was no possibility of the introduction of extraneous substances in transit from one laboratory to the other since the pigs were all carried in perfectly clean containers, kept isolated from the general laboratory stock, and killed in less than 2 hours after arrival.

The remarkable diminution in cellularity and replacement by fatty tissue observed in the Group III marrows suggested a fairly pronounced liberation and delivery of blood elements. This has been borne out to a certain extent by the consistent rise in peripheral red blood cell levels noted by Jacobson [12] (see his Table III). The abrupt appearance of large numbers of mature erythrocytes, the numerous islands of normoblasts, the ragged remnants of the islands of hemic precursors, and the marked diminution of megaloblasts created the justifiable impression that some factor had produced a rapid maturation of the cellular marrow and extrusion of the end elements. The

sudden impetus to maturation of erythroid elements evidently had much to do with the lessened numbers of eosinophiles and megakaryocytes noted. These returned to their previous levels in the later groups after acceleration had lessened.

The 2 guinea pigs of Group IV, in themselves, offered insufficient data for conclusive interpretation but coupled with the data obtained from the other groups they served as a definite connecting link. Their status will be considered in connection with that of

TABLE III

Relative Prominence of Significant Features of Bone Marrow Variation

Group	I	II	III	IV	V
Immature cells	++++	++++	++	++	++
Megaloblasts	+++	+++	+	+	++++
Mitotic figures	+	+	+	++++	++
Normoblasts	++	++	++++	++++	++++
Erythrocytes	+	+	++++	++++	++++
Megakaryocytes	++++	++++	+	++	++++
Eosinophiles	++++	++++	++	+++	++++
Cellularity	++++	++++	+	++	+++
Fat vacuoles	+	+	++++	++	+++

Group V. The relatively bizarre appearance afforded by the marrows obtained in Group V was at first inexplicable and offered no well defined basis for any conclusion. Areas of relative hypoplasia containing many erythrocytes, normoblasts, and mature granulocytes similar to those in Group III were adjacent to areas of dense cellularity resembling those of Groups I and II. These cellular regions differed, however, in that they contained abundant mitotic figures and both normoblasts and erythrocytes were present in as large numbers as had been observed in the liver response series. Despite apparent focal similarity to Groups I and II this group exhibited no impedance of maturation.

What then was the underlying difference? Groups I and II

possessed marrows filled with immature cells affording them a powerful blood-forming potentiality, the impetus for which was evidently deficient. Adequate maturation for the animals' needs occurred but there remained a large reserve which under the conditions of the experiment was stable. The stimulating factor was obviously contained within the liver extract, for the administration of a small dose of this substance was sufficient to produce a rapid increase of mature elements, depletion of the immature, and readily descried fat replacement. Continued administration of similar doses on sequential days produced in Group IV, not the expected progression of marrow depletion in the long bones, a state that would complete the analogy to pernicious anemia, [24] but a reversion to the type of marrow observed in Group V.

This last group possessed marrows that showed unquestionable evidence of unhampered maturation. Areas of relative hypoplasia were presumed to represent recently depleted storage spaces, whereas those retaining intense cellularity in which, however, maturation was advanced, were considered ripe hemic reservoirs from whence delivery was available in an irregular focal sequence. In much the same manner as the kidney and other secretory units of the living organism exhibit intermittent activity, the hemopoietic system has shown considerable evidence of a similar functional quality. The work of Doan and others [7, 25] gives ample evidence of such cyclic activity.

Definite differences having been observed in the several marrow groups it remained only to determine the factor or factors underlying these variations. The age, stock, sex, weight and environment were essentially the same in all the guinea pigs. The only obvious variation in the experimental conditions was one of diet. Groups I to IV had received oats, carrots and lettuce, with the addition of liver extract in Groups III and IV, and Group V was fed hay, oats and carrots. Investigation of Jacobson's data revealed the fact that in his earlier investigations he had found that animals transported in hay-lined containers and kept in similarly lined bins had been found unsatisfactory for assay purposes because of spontaneous rises of reticulocytes. These animals removed to wire cages and placed on a hay-free diet gradually assumed stable reticulocyte levels and responded satisfactorily to liver extract. Similarly, consistently reacting pigs when permitted to eat hay in addition to their basic diet

rapidly became unstable. No attempt is made to explain the small group of guinea pigs that showed unsatisfactory reticulocyte responses even under the prescribed regimen over a prolonged period. The marrow of none of these animals was studied.

Whether it is the food substance or vitamin content of the hay, the constituents of the molds growing in it, or the element of coprophagy, uncontrolled in Group V, that constitutes the source of the bone marrow stimulating factor is not fully established.[2, 22, 30]

Nor is it definitely known whether any inhibitory factor exists in lettuce. The fact remains, however, that these are the only perceptible conditions in Group V at variance with those in Groups I and II. It is permissible, therefore, in view of the absence of other effective possibilities to conclude that it is the dietary variation that is basic in the production of the hyperplastic, incompletely maturating marrow of the guinea pigs in Groups I and II. The addition of liver extract to the dietary regimen unquestionably produces release from the maturation defect. Continued administration of liver extract permits the marrow to revert to the characteristic unhampered maturation of the uncontrolled pigs in Group V. Cellularity is regained but the proportion of ripened elements is manifestly normal, using as the basis for normality the animals of Group V. The large number of macrophages observed in 1 pig of Group IV very probably was resultant on removal of disintegrating fat occurring in the course of replacement by active tissue. It is also plausible that failure to eliminate completely all sources of reticulocytogenic substances will readily serve to obviate the fundamental purpose of the dietary restriction, the inhibition of hemopoietic maturation.

Summary and Conclusions

1. Five groups of guinea pigs were studied for the purpose of observing the effect of liver extract on the bone marrow and to determine the mechanism of its action.

2. Ten animals fed a diet of oats, carrots and lettuce showed densely cellular marrow with evidence of depressed maturation of its elements.

3. Seven pigs fed the same diet exhibited marked depletion of cellular content with abruptly accelerated maturation following the administration of a single dose of liver extract.

4. Two pigs fed a similar diet manifested return of cellularity but

persistence of maturation subsequent on the administration of daily doses of liver extract for 6 and 10 days respectively.

5. Twenty pigs from the ordinary laboratory stock fed only hay, oats and carrots possessed a relatively densely cellular marrow which showed no inhibition of maturation.

6. Small and varying numbers of megaloblasts were identified within the marrows of pigs from all 5 groups.

7. The diet of oats, carrots and lettuce obviously lacked a substance available in both liver extract and a diet of hay, oats and carrots. This substance permitted maturation of immature hemic elements.

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DESCRIPTION OF PLATES

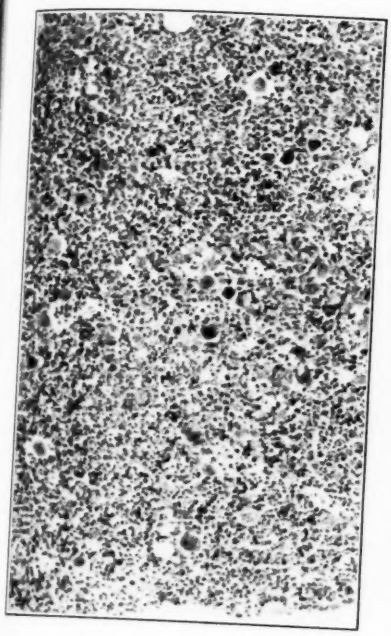
PLATE 93

FIG. 1. Femoral marrow from a guinea pig of Group I. This animal was fed a diet of oats, carrots and lettuce, the prescribed Jacobson regimen. Note the dense cellularity, large numbers of megakaryocytes and scarcity of fat vacuoles. × 100.

FIG. 1*a*. A similar section at higher power. The large numbers of immature cells, the scantily scattered normoblasts, and the sparseness of mature erythrocytes and granulocytes are evident. Stained with azure II eosin. × 650.

FIG. 2. Femoral marrow from a guinea pig of Group III. This animal was fed a diet of oats, carrots and lettuce. A single test dose of liver extract was given and the pig killed at the peak of the reticulocyte response. There is a pronounced increase in the amount of fat present and many large islands of normoblasts may be observed. × 100.

FIG. 2*a*. A high power view of a marrow section from a pig of the same group. An island consisting wholly of normoblasts is seen. At its periphery are 2 megakaryocytes and an irregular fringe of mature erythrocytes. Stained with azure II eosin. × 650.



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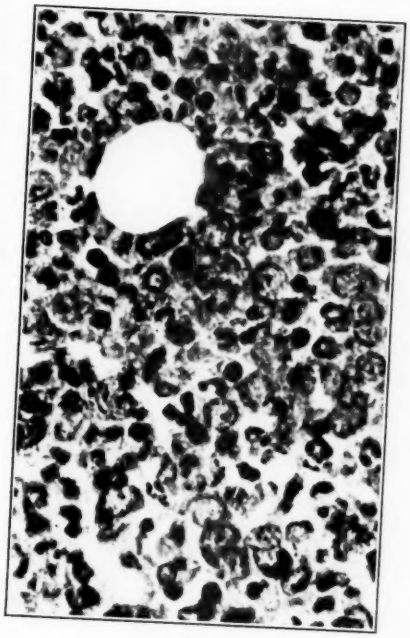
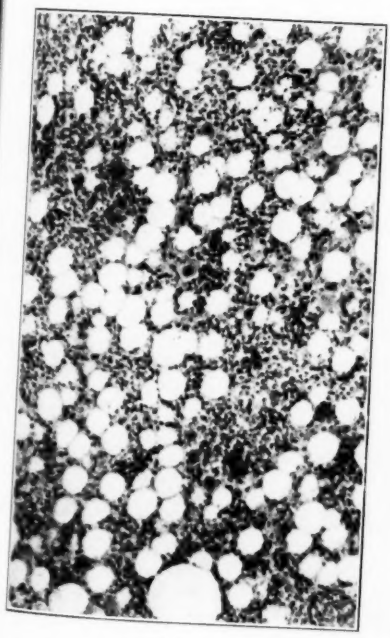


1 *a*



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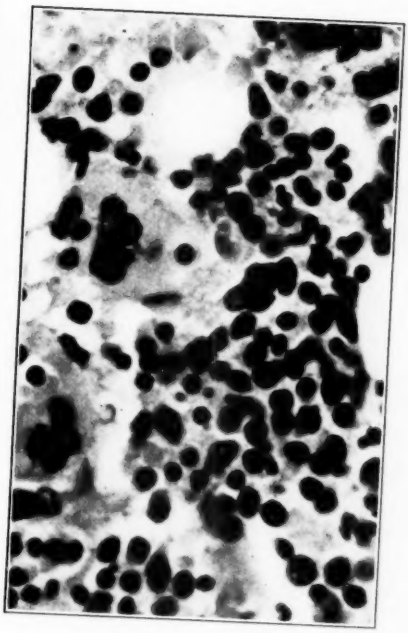


2 *a*

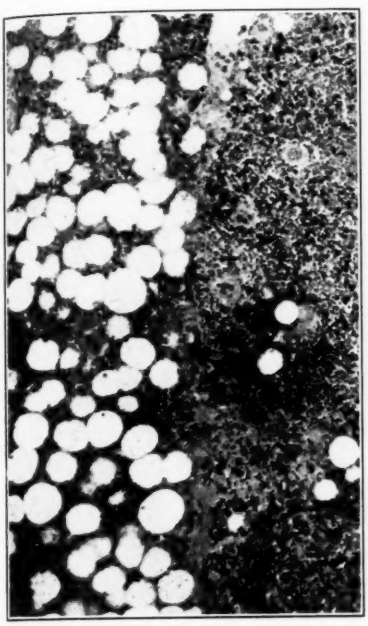
Response of Bone Marrow to Liver Extract

PLATE 94

FIG. 3. Femoral marrow from a pig of Group V. This group was fed hay, oats and carrots and was kept in the laboratory stock farm under uncontrolled conditions. The variation in cellularity of adjacent areas is well demonstrated: the cellular area exhibits fairly large numbers of loosely aggregated normoblasts. × 100.

FIG. 3*a*. A high power view of the cellular portion of the same section showing the intermixture of immature and relatively mature cells with no evidence of inhibition of maturation. Stained with azure II eosin. × 650.

FIG. 4. Drawing of an oil immersion field in an imprint preparation from the femur of a pig in Group V. Two megaloblasts (*a*) show the shreddy arrangement of the chromatin and in a faint manner the scroll-like parachromatin. The appearance of the nuclei is distinctly different from the stippled arrangement observed in the myeloblast (*b*). Progressive maturation produces coarsening as represented in the late erythroblast (*c*) and the lumpy pyknotic chromatin of the normoblast (*e*). Free nuclei (*d*) preclude identification. Wright-Giemsa Stain. × 1100



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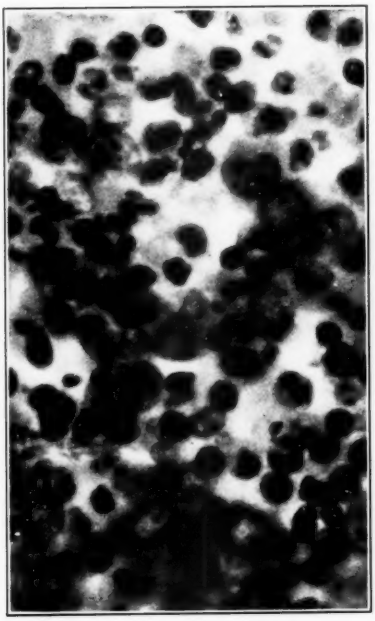
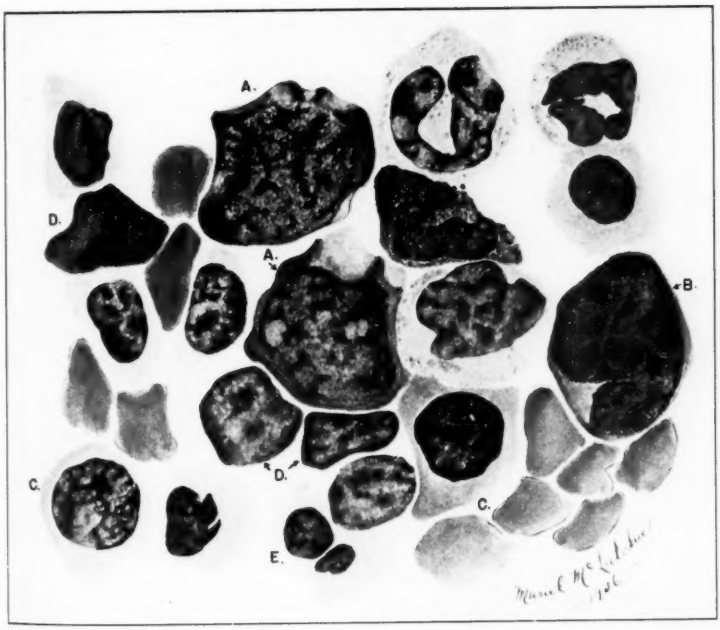


3 a



4

THE PATHOGENESIS OF CORTICAL NECROSIS OF THE KIDNEYS IN RABBITS FOLLOWING THE INJECTION OF STAPHYLOCOCCUS TOXIN *

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There is general agreement that necrosis of the renal cortex is the most constant pathological change occurring in rabbits following the injection of staphylococcus toxin. The mechanism of its production, however, has led to some dispute. Neisser and Wechsberg [1] held that it is an anaemic necrosis secondary to thrombosis of the vessels in this region. VonGlahn and Weld [2] accounted for the tubular necrosis by an interference with glomerular circulation, not so much by thrombosis as by haemorrhage resulting from damage to capillary loops. On the other hand, Rigdon,[3] while describing haemorrhage as a conspicuous feature, believed that the necrosis is the result of a direct action of the toxin on tubular epithelium.

Since necrosis is invariably accompanied by haemorrhage and some degree of thrombosis in the later stages, it becomes necessary to study the earliest changes in order to separate these factors. The present study was directed toward this end by investigating new evidence; namely, early changes in the mitochondria of the tubules.

Previous workers have commented on the wide variation in susceptibility of individual rabbits to staphylococcus toxin. Rigdon, Joyner and Ricketts,[4] for example, found that some animals died immediately after the intravenous injection of 0.5 cc. of their toxin while others received as much as 22.5 cc. over a period of 9 days before death occurred. In the light of recent knowledge concerning the toxin, however, much of this variability can be accounted for and reproducible results obtained. At least two gross errors can be controlled.

First, a normal adult rabbit selected at random may be entirely unsuitable for this type of experiment. Ramon, Richou and Descazeaux[5] have shown that a considerable proportion of normal rabbits contain "natural" antitoxin in their blood serum. This observation

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has been confirmed by Roy [6] in this laboratory for the stock from which the animals in this study were obtained. Therefore, all animals used in this study were selected on a basis of preliminary titrations of their serums.

Second, the potency of the toxins used by previous workers have not been expressed in terms of any defined unit. VonGlahn and Weld [2] have, to some extent, avoided this error by selecting a toxin which, on a basis of previous experience, was known to produce kidney lesions in amounts ranging from 0.1 cc. to 0.5 cc. In the present study toxins were selected that possessed a definite measurable potency in terms of haemolytic activity. The unit employed will be defined subsequently. In a normal rabbit which has no demonstrable "natural" antitoxin, kidney lesions are invariably produced by such a toxin in amounts as low as 0.01 cc.

It is recognized that the measurement of a toxin in terms of its haemolytic content does not necessarily provide information regarding the substance responsible for the kidney lesions. There are at least six well recognized activities of staphylococcus toxin which have been demonstrated repeatedly: haemolytic, leukocytic, dermonecrotic, enterotoxic, plasma coagulating and acute lethal. Burnet [7] and Gengou [8] maintain that a single toxin may exhibit various activities according to the conditions of the experiment. Bigger,[9] Glenny and Stevens,[10] Weld and Gunther [11] and Forssman,[12] present evidence to show that staphylococcus filtrates are mixtures of several kinds of toxins, each fraction being, to some extent, separable from the others. The majority of recent work apparently supports the latter viewpoint. If this be accepted there is no theoretical reason why the measurement of haemolytic activity should predict a content of "nephrotoxic" substance in any given toxin. Practically, however, it has been found in this study that a measurement of haemolysin provides a convenient unit by means of which reproducible results may be obtained provided the animals are selected with a knowledge of the presence of "natural" antitoxin. Again, this "natural" antitoxin is measured in terms of antihaemolytic power.

The present work is not intended to support the theory of the unity of staphylococcus toxins. A personal viewpoint, in fact, favours the conception of separate and distinct fractions and it is probable that the strains of staphylococcus employed were capable

of elaborating several toxic fractions. The measurement of haemolysin does not necessarily imply that it is the substance responsible for the kidney lesions, merely that both haemolysin and kidney necrosing substances are elaborated in amounts showing a sufficiently regular ratio, so that the more easily measured haemolysin is some index of the quantity of the other fraction.

Methods and Materials

Animals: Thirty-two normal rabbits weighing from 1300 to 3000 gm. were used. The serum of each was titrated for its antihaemolytic content previous to the injection of toxin. Twenty-nine of these contained no demonstrable antihaemolytic substance; 3 contained more than enough to neutralize the amount of toxin used.

Bacteria: Three strains of *Staphylococcus pyogenes aureus* were used. One was cultured from a human furuncle, a second from a blood culture in a case of fatal staphylococcus pyemia, the third was obtained through the courtesy of Dr. E. O. Jordan as a representative of the enterotoxic strains of staphylococci (Dr. Jordan's No. 5).

Toxin: The medium employed was semisolid nutrient agar (0.3 per cent agar) at a pH of 7.2 poured into 15 cm. Petrie dishes in 100 cc. amounts. The staphylococci were heavily seeded from a young agar slant into the semisolid agar which was incubated at 37° C. for 72 hours in an atmosphere of 20 per cent carbon dioxide and 80 per cent oxygen. The whole culture was then centrifuged and the supernatant fluid sterilized by filtration through a Seitz EK filter. This filtrate containing the toxin usually has a pH of about 8.0 and, if sterile, will maintain its haemolytic power for several weeks with very little loss when stored at 4° C. The filtrate was titrated and used exactly as it was obtained.

Units: The unit of toxin employed has been defined by Burnet.[13] It is the least amount of a toxic filtrate that will produce 50 per cent haemolysis of 1 per cent washed rabbit cells in a volume of 1 cc. in 1 hour at 37° C. This unit has a fairly constant relation to the standard international unit of antitoxin (Hartley and Smith [14]) within an error of 10 to 15 per cent for various batches of toxin. The average lethal dose for normal rabbits whose serum contains no demonstrable antitoxin is about 300 plus or minus 50 Burnet haemolytic units per kilogram.

Procedure: Most of the animals were given a single intravenous injection; a few received several intradermal injections followed by a single intravenous injection 2 days later. Twenty-two rabbits received a sublethal dose (100 to 300 units) of toxin and were killed at intervals afterward. Five received a sublethal dose; the left kidney was removed under sodium amytal anaesthesia after 2 hours and the animal was allowed to live 24 to 48 hours before being killed to remove the right kidney. Two animals were anaesthetized: the upper pole of the left kidney was biopsied, a sublethal dose of toxin was injected intravenously and the left kidney removed after 5 minutes; the upper pole of the right kidney was biopsied after 30 minutes and the animal was killed in 45 minutes. Three animals whose serum contained more than sufficient antitoxin to neutralize a lethal dose of toxin were given a sublethal dose and killed at 30 minutes, 3 hours and 24 hours respectively.

Autopsies: A complete autopsy was performed on each animal. This report, however, limits itself to kidney changes.

Sections: Duplicate tissues were fixed in Zenker's fluid without acetic acid for general histology, and in Regaud's fluid for the study of mitochondria. Paraffin embedding was employed. The staining methods used routinely on Zenker-fixed material were: haematoxylin and eosin; haematoxylin, acid fuchsin and picric acid; azocarmine, aniline blue and orange G. It was found that sharper differentiation of glomerular basement membranes in the aniline blue technique was obtained by slightly modifying McGregor's [15] formula. Following the azocarmine stain the tissue was mordanted 24 hours in 5 per cent phosphotungstic acid, washed slightly in water and stained 24 hours in aniline blue-orange G. The section, heavily overstained by this procedure, was then differentiated, without washing in water, in dilute alkaline alcohol (1 cc. of 5 per cent sodium hydroxide per 100 cc. of 95 per cent alcohol) for 15 seconds, flooded with dilute acetic alcohol (1 cc. of glacial acetic acid per 100 cc. of 95 per cent alcohol) until the blue returned (about 2 or 3 seconds) then dehydrated, cleared and mounted as usual.

Mitochondria were stained by Masson's [16] method with warmed aniline acid fuchsin and differentiated in dilute picric alcohol after 4 days fixation in Regaud's fluid followed by 6 days in 3 per cent potassium dichromate.

Pathological Findings

Gross Changes: In no instance were gross lesions visible in the kidney within 1 hour after injection, but after 1 hour numerous small, irregularly shaped areas of redness are seen on the surface. The capsule strips cleanly from these areas leaving no bleeding points. On section these areas are seen to be confined to the cortex.

In 2 to 3 hours frank haemorrhage is seen. The surface of the kidney is mottled with irregularly shaped, deep purple areas of haemorrhage ranging in size from pin-point to 2 or 3 mm. The capsule strips readily leaving bleeding points which show that the larger haemorrhagic areas are subcapsular extensions of smaller haemorrhages. On section the haemorrhage is seen to be confined to the cortex and it is most extensive near the capsule.

In 4 or 5 hours larger areas of haemorrhage are seen. They are still confined to the cortex and are most abundant near the capsule. A section at right angles to the long axis of the kidney shows that these haemorrhages are more or less segmentally arranged. That is, regions of haemorrhage extend from the cortico-medullary junction to the capsule in a linear arrangement without interruption and the kidney tissue between appears normal.

After 5 hours the linear distribution of haemorrhage becomes less distinct as the entire cortex is engorged with blood. On stripping the capsule very fine fibrinous adhesions are occasionally broken.

In 12 to 18 hours small yellowish white areas are seen on the surface from which the capsule strips readily. On section these areas are often wedge shaped and invariably are surrounded by a haemorrhagic border.

After 24 hours the white areas extend and tend to coalesce. If the animal survives 7 to 8 days, the maximum damage is seen. Such kidneys exhibit a completely white bloodless cortex over the entire surface. On section the white area is seen extending to the subcortical region where it is sharply demarcated from the medulla by a zone of haemorrhage.

Microscopic Changes: In contrast to the gross picture definite changes are seen microscopically within the first 5 minutes. The first change is a dilatation and engorgement of the arterioles and glomerular capillaries. It is not extensive 5 minutes after the injection of the toxin but is undoubtedly different from the normal and

histologically identical with the appearance of such glomeruli at a later stage. The mitochondria of the convoluted tubules in the vicinity of dilated glomeruli show evidence of damage in that many of them become fragmented and tend to assume a spherical shape rather than the normal rod shape. Sections stained by routine methods show no other change in cytoplasm or nuclei at the 5 minute interval.

In from 30 minutes to 1 hour dilated glomeruli become increasingly numerous and the tubular mitochondria are more severely damaged. Definite haemorrhage is uncommon at this stage.

In from 1 to 3 hours mitochondria are reduced to a few globules in the cells of the tubules surrounding dilated glomeruli. Many of these tubules show no mitochondrial substance at all (see Figs. 8 and 9). In other areas of the section where glomeruli are not dilated the tubular mitochondria appear as normal rod shaped filaments. About this time glomerular dilatation appears to be maximal. By following the cortex around the entire extent of a section cut through the whole kidney a pattern of alternating dilated and normal glomeruli may be seen. That is, the dilatation does not appear to be irregularly distributed among the glomeruli but rather in a segmented arrangement. The whole depth of cortex from corticomedullary junction to capsule in one area shows every glomerulus containing dilated capillaries while immediately beside this region every glomerulus through the entire depth of the cortex appears normal. Thus the whole cortex presents a pattern of alternating segments of dilated or normal glomeruli. Figure 1 is a microphotograph taken at the border between two such segments.

At 3 hours some of the glomerular capillaries are enormously dilated, a single loop may be stretched enough to push the remainder of the tuft into a small area of the glomerular space. Such widely dilated loops are engorged with red cells and stained with haematoxylin-eosin and may easily be mistaken for haemorrhage into Bowman's capsule. With the aniline blue stain, however, the basement membrane of such a loop is seen to be intact although greatly thinned out. Figure 2 illustrates this point. In other glomeruli the basement membrane has ruptured and extravasation of red cells occurred.

Haemorrhage becomes more pronounced during the 3 to 4 hour period. It is most conspicuous in the vicinity of a glomerulus

which itself is engorged with extravasated cells. In many of these glomeruli no remnants of the glomeruli tuft are seen; in others the tuft is reduced to a shrivelled stump at the point of entrance of the afferent vessel while the rest of Bowman's space is filled with red cells (see Fig. 3). The convoluted tubules in this region are either devoid of mitochondria or contain a few globules of various sizes; none is in the form of rod shaped filaments. With routine stains the tubular cells appear swollen and frequently contain hyaline droplets but the nuclei are usually well preserved except in regions where the tubules are definitely necrotic. Fibrin is uncommon but does occur in some of the arterioles and occasionally fine strands are found in haemorrhagic glomeruli.

In from 5 to 8 hours haemorrhage becomes more extensive and necrotic tubules more numerous. In some regions dilated glomerular tufts persist without rupturing and, here, the epithelial cells of Bowman's capsule are altered. Instead of the flat cells with oval nuclei closely applied to the capsular basement membrane normally seen, at this stage one finds cells with swollen cytoplasm bulging into the capsular space and the nuclei are rounded and stain either poorly or very intensely. The latter cells appear to be proliferating. Albuminous deposits are common in the capsular space. In the tuft the perivascular epithelial cells greatly outnumber the endothelial cells and, when the capillary loops are distended, both show some nuclear fragmentation.

In 24 to 48 hours haemorrhage is maximal. It is most conspicuous at the periphery, in the region of ruptured glomeruli (see Fig. 4).

Although necrosis is seen in occasional tubules as early as 5 hours, it does not become extensive until 24 hours. Beginning at the periphery of the cortex and gradually advancing toward the corticomedullary junction the necrotic zone is always preceded by an area of haemorrhage. Sometimes this haemmorhage outlines a wedge shaped region of necrosis most commonly seen at about 24 hours. After 48 hours these isolated areas tend to coalesce so that eventually the entire depth of cortex is necrotic and a sharp line of haemorrhage separates it from the medulla (see Fig. 5).

Within the necrotic zone the architecture of the glomeruli and tubules is well preserved even when the cells contain no stainable nuclei. Invasion by inflammatory cells is slow although a few polymorphonuclears are seen as early as 24 hours. Even at 5 to 8 days,

when the entire cortex is necrotic, the inflammatory reaction appears to be in its early stages. The invading cells arrive from two regions; from the zone of haemorrhage at the junction of cortex and medulla and from the renal capsule at the periphery, the latter apparently by way of the vessels of the capsule itself. The advancing edge of inflammatory cells always shows pyknotic and fragmenting nuclei as well as cellular débris, the more healthy looking cells being farther back near the blood supply.

Discussion

The present study shows that the earliest demonstrable change in the kidneys of rabbits receiving an intravenous injection of staphylococcus toxin is a dilatation of blood vessels. This confirms the experience of previous investigators who, however, have not described it as early as 5 minutes after injection. The toxin apparently exerts its first effect on small vessels, as is shown by the dilatation of capillary loops. But the effect must extend farther back in the vascular tree than capillaries, because damage to capillary endothelium alone, while it might result in loss of tone and dilatation of glomerular loops at normal arterial pressure, does not account for the segmental distribution of these dilated glomeruli. In a given segment all of the capillaries are dilated, in another none. If one assumes that arterioles and smaller arteries are primarily involved this segmental pattern becomes reasonably explained. Presumably the small arteries are dilated and all of the glomeruli supplied by them respond to the increased blood flow by dilatation.

The capillaries themselves are also affected by the toxin. The evidence for this is found in the frequent extreme dilatation of a single loop and this is clearly demonstrated in the sections stained by aniline blue (Fig. 2). This stain also provides a means of understanding the mechanism of haemorrhage. Such greatly dilated loops eventually rupture, apparently with explosive force, because another stage is found (Fig. 3) in which the glomerular tuft is reduced to a shrunken residue of frayed ends. Bowman's space is engorged with red cells and red cells fill the lumen of the corresponding tubule.

The toxin exerts a direct effect on the tubular epithelium at an early stage. The evidence for this is found in mitochondrial changes which precede haemorrhage. As early as 5 minutes after the injection of toxin mitochondria begin to lose their structure as rod

shaped filaments, becoming fragmented and globular. This change is more marked in 1 to 3 hours, at which time tubules in the vicinity of dilated glomeruli which have not yet produced haemorrhage often contain no mitochondrial substance at all or merely a few globules (Figs. 8 and 9). At this stage routine haematoxylin and eosin stains usually show no alteration in nuclei or cytoplasm.

Five to 6 hours later the tubular epithelium exhibits changes visible by ordinary stains. The cells are swollen, contain hyaline droplets, nuclei are pyknotic and necrosis is evident. At this time also the epithelium of Bowman's capsule shows evidence of reaction to the toxin by similar changes. Probably this capsular epithelium has been affected at a much earlier stage, since embryologically it is the same type of cell that occurs in the convoluted tubule, but it lacks mitochondria by means of which early changes might be detected.

It might reasonably be expected that if the toxin exerts such damage to renal epithelium in the absence of haemorrhage it must be excreted by the kidneys and therefore be detectable in the urine. This is rarely possible because such small doses are used — 100 to 300 Burnet haemolytic units. Some of the injected toxin no doubt is absorbed by other body cells; some of the amount filtered through the glomeruli is probably concentrated in the tubules and absorbed by them and the final excretion must be so low in toxin that it remains undetectable. Theoretically, much of the toxin is absorbed in the tissue, but even if the total amount injected were excreted in the urine and thereby diluted only 100 times it would not be detectable.

On the other hand, the use of large amounts of toxin defeats this type of experiment. When 10,000 to 50,000 haemolytic units are given intravenously toxin may sometimes be detected in the urine, but the animal dies quickly, usually within 2 or 3 minutes. The problem is open to experiment by the use of formalized toxin and a measurement of the urine's combining power with antitoxin, or by perfusion methods.

The later changes in the kidney leading to large necrotic areas have been adequately described by previous investigators, but one point deserves further comment; the slowness of polymorphonuclear invasion in the necrotic area. Two possibilities suggest themselves: first, the toxin as such may not be so markedly pyogenic as living staphylococci; second, a more reasonable explanation, the toxin is

small in amount, widely absorbed by susceptible cells and not diffusible to a sufficient extent to be pyogenic. From the latter viewpoint the inflammatory reaction may be a response mainly to sterile necrotic tissue.

Controls for these experiments were the animals whose serum contained an excess of antitoxin. By this means comparable amounts of the same batch of toxin were used rather than injections of sterile broth. For example, rabbit No. 440 (Figs. 6 and 7) whose serum contained 640 Burnet antihaemolytic units (approximately 1 international unit of antitoxin) was injected with 260 Burnet haemolytic units of toxin J5-2 and 3 hours later showed no injury to the kidney. Rabbit No. 437 (Figs. 8 and 9) contained no detectable antitoxin in its serum, was given 300 Burnet haemolytic units of toxin J5-2, a comparable dose since the average lethal dose is not measurable within a limit of 50 units, and 3 hours later showed marked injury to the kidney. Such results indicate that the injury is due to the toxin and not to other pharmacologically active substances in the medium which might be elaborated during the incubation of the cultures.

Conclusions

The sequence of events may be reconstructed as follows: The toxin causes a dilatation of small arteries, arterioles and capillaries (Figs. 1 and 2). It is excreted through the glomeruli and causes direct damage to tubular epithelium (Fig. 9). It injures capillaries, producing loss of tone with subsequent extreme dilatation and eventually sudden rupture (Fig. 3). The sudden bursting of glomerular loops is the mechanism by which haemorrhage occurs (Fig. 4). Necrosis quickly follows for the tubular epithelium already damaged by the toxin has little resistance to the anaemia resulting from haemorrhage (Fig. 5). The necrosis is sharply limited to the cortex. Inflammatory cells are slowly mobilized and enter the necrotic area both from the medulla and from the vessels of the renal capsule.

Summary

1. Attention is called to two errors in previous studies on the effect of staphylococcus toxin on rabbit's kidneys. These are: (*a*) the failure to select animals on a basis of preliminary titrations of their serums, many of which possess considerable quantities of

"natural" antitoxin; and (*b*) the failure to measure toxin in terms of some unit of activity.

2. Evidence based on mitochondrial changes in tubular epithelium is presented favouring the view that staphylococcus toxin exerts a direct effect on the kidney cells separate from the changes secondary to haemorrhage.

3. The vascular damage reported previously has been confirmed and the mechanism of haemorrhage described.

4. Cortical necrosis is eventually the result of two factors: (*a*) direct injury to cells by toxin; and (*b*) anaemia resulting from haemorrhage.

NOTE: I am greatly indebted to Prof. E. G. D. Murray for his encouragement and criticism throughout this work. I also wish to thank Mr. William Clark for the microphotographs.

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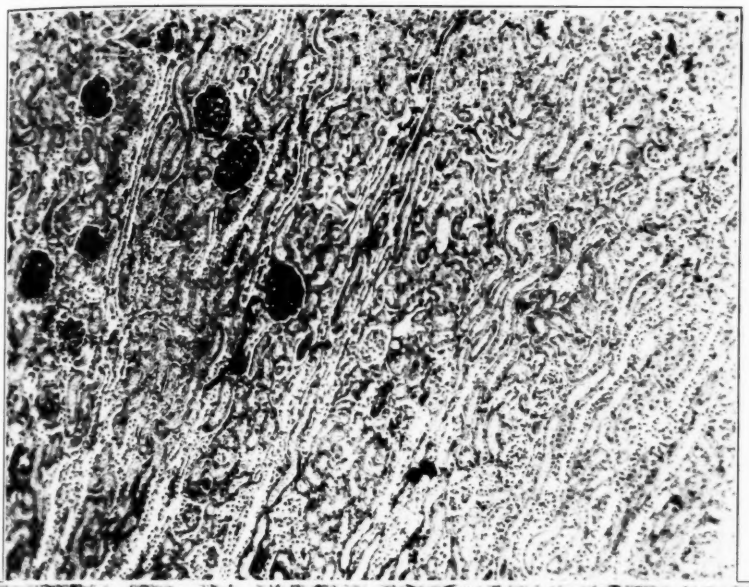
DESCRIPTION OF PLATES

PLATE 95

FIG. 1. The border between two segments of dilated and normal glomeruli 3 hours after the intravenous injection of 300 units of toxin. Haematoxylin-acid fuchsin-picric acid stain. × 70.

FIG. 2. Untouched microphotograph of a widely dilated capillary loop in a glomerulus 3 hours after the intravenous injection of 300 units of toxin. Note that the dilated loop has pushed the rest of the tuft to one side. The basement membrane is thinned out but still intact. Azocarmine-aniline blue-orange G stain. Photographed through Wratten 25 filter. × 270.

FIG. 3. Rupture of a glomerular capillary 3 hours after the injection of 300 units of toxin. Bowman's space is engorged with extravasated red cells and the frayed ends of glomerular basement membrane are barely visible. Azocarmine-aniline blue-orange G stain. Wratten 25 filter. × 270.



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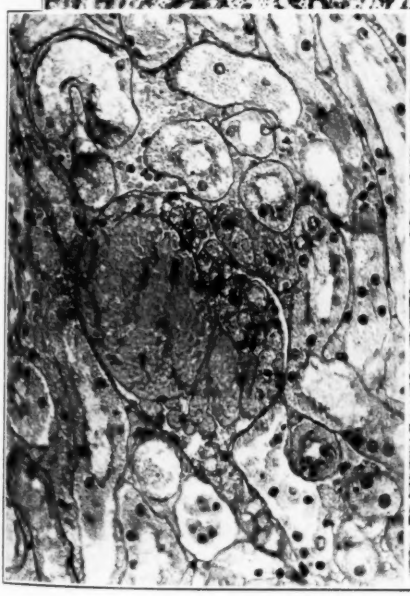

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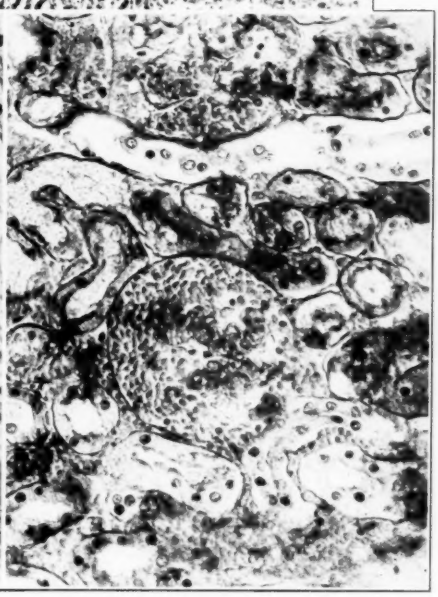


3

Glynn

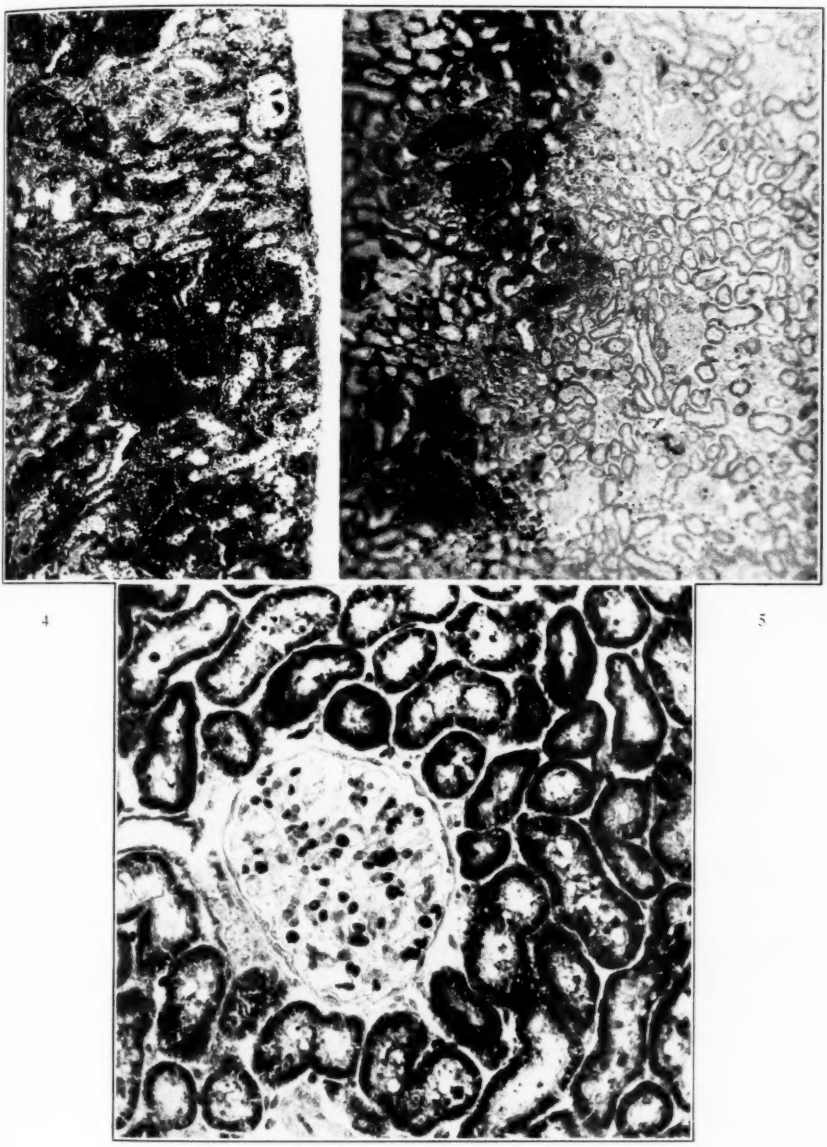
Cortical Necrosis of Kidneys

PLATE 96

FIG. 4. Haemorrhage into the cortex 48 hours after the intravenous injection of 200 units of toxin. The haemorrhage originates in the glomeruli. Haematoxylin-acid fuchsin-picric acid stain. × 70.

FIG. 5. Necrosis of cortex 8 days after the intravenous injection of 150 units of toxin. The cortex is completely bloodless and necrotic. The architecture of the region is preserved although it contains no stainable nuclei. The zone of necrosis is sharply separated from the medulla by an area of haemorrhage. There is beginning invasion by polymorphonuclear cells. Haematoxylin-acid fuchsin-picric acid stain. × 70.

FIG. 6. Mitochondria of tubules 3 hours after the intravenous injection of 260 units of toxin in a rabbit whose serum contained 640 units of antitoxin. The glomerulus is not dilated and the mitochondria of the tubules are normal. Regaud fixation, Masson's acid fuchsin-picric alcohol stain. × 270. Compare with Figure 8.



4 5

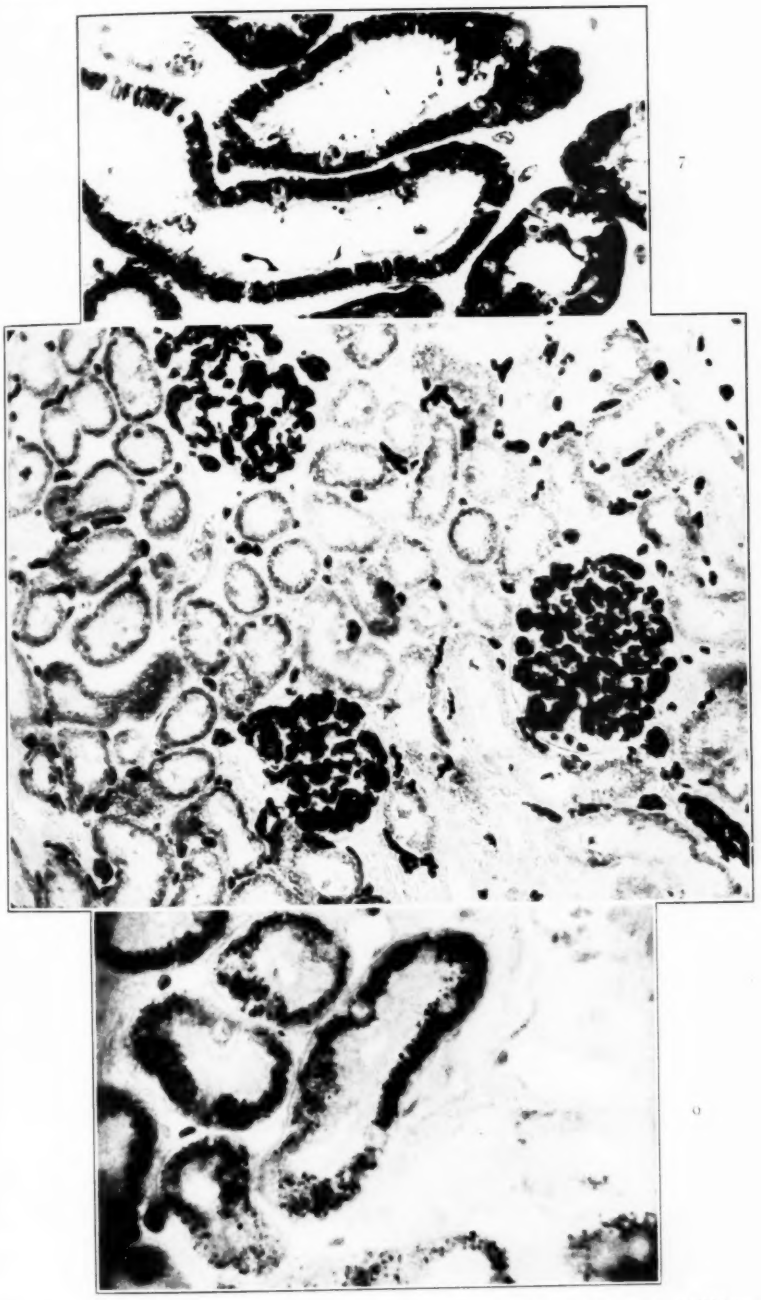
6

PLATE 97

FIG. 7. Same as Figure 6. Detail of mitochondria which appear as normal rod shaped filaments. × 800. Compare with Figure 9.

FIG. 8. Mitochondria of tubules 3 hours after the intravenous injection of 300 units of toxin in a rabbit whose serum contained no antitoxin. The glomeruli are dilated and the mitochondria reduced to small globules. Regaud fixation, Masson's acid fuchsin-picric alcohol stain. × 270. Compare with Figure 6.

FIG. 9. Same as Figure 8. Detail of mitochondria which are fragmented and globular. A tubule in the lower part of the figure contains no mitochondria. × 800. Compare with Figure 7.



Glynn Cortical Necrosis of Kidneys

SCIENTIFIC PROCEEDINGS OF THE THIRTY-SEVENTH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

HELD AT NORTHWESTERN UNIVERSITY,

CHICAGO, ILLINOIS

MARCH 25 AND 26, 1937

BUSINESS MEETING

OF

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Held at Thorne Hall, McKinlock Campus,

Northwestern University,
Chicago, Illinois

March 26, 1937

PRESIDENT FOOT PRESIDING

The Secretary presented the nomination of the Council for officers as follows:

President	ESMOND R. LONG
Vice-President	EARLE B. MCKINLEY
Treasurer	FRANK B. MALLORY
Secretary	HOWARD T. KARSNER
Incoming Member of Council	SAMUEL R. HAYTHORN
Assistant Treasurer	FREDERIC PARKER, JR.
Assistant Secretary	ALAN R. MORITZ

Voted unanimously to elect those nominated.

Voted to elect the following new members:

Leo Alexander	Harold M. Dixon
Frank C. Andrus	William E. Ehrich
D. Murray Angevine	Harry C. Fortner
Herman Bolker	Edwin S. Gault
G. John Buddingh	David M. Grayzel
John C. Bugher	John C. Grill
Edward L. Burns	Cornelius S. Hagerty
Albert DeGroat	Ernest B. Hanan

Edward H. Hatton
Louis M. Hellman
Sheldon A. Jacobson
Paul Kimmelstiel
Cecil A. Krakower
Richard J. Lebowich
Amour Liber
Louis Lichtenstein
John W. Miller
Donald A. Nickerson
Edgar H. Norris
Peter Olafson
Antonio Rottino
Herbert J. Schattenberg
Arnold F. Strauss
Calvin Torrance

It was also voted to reinstate Drs. H. H. Bullard, J. F. Rinehart and H. M. Zimmerman.

Voted to accept with regret the resignations of Drs. F. B. Gurd, H. Moak, R. Muir, S. T. Orton, F. E. Sondern, R. M. Taylor, J. C. Torrey and M. Wollstein.

Voted to record with deep regret the deaths of Drs. H. M. Adler, B. deVecchi, C. P. Howard, M. Rothschild and F. R. Zeit.

Voted to elect Lt.-Col. George R. Callender as delegate to Congress of American Physicians and Surgeons, and Dr. Ward J. MacNeal as alternate.

The Secretary announced that the next meeting of the Association will be held in Atlantic City, New Jersey, in May, 1938, in conjunction with the Congress of American Physicians and Surgeons.

In discussion of the Symposium for 1938 the Secretary pointed out that because the Congress of American Physicians and Surgeons will utilize the time of one session, the sessions of this Association will be only three in number. Owing to the fact that the number of contributions to the program is increasing each year, the inclusion of a Symposium might be a severe restriction on the number of papers.

Voted to have no Symposium in the 1938 meeting.

The Secretary drew attention to the fact that the Constitution is published with the list of members, and read the following By-Laws which were adopted at the time of organization of the Association.

BY-LAWS

1. There shall be an annual meeting at such time and place as the Council shall determine.
2. At the first meeting of the Association a Council of Seven shall be chosen, one of whom shall go out of office annually and shall not be immediately eligible for reelection.
3. The Council shall, immediately after its election, determine by lot the terms of office of its members.
4. The vacancy created by the annual retirement of a member of the Council shall be filled by nomination by the Council and election by the Association, and the individual thus elected shall serve for a term of seven years.
5. Should a vacancy occur in the Council, otherwise than by the expiration of the term of service, the Council may elect a member to serve for the unexpired portion of the term.
6. The Annual Dues of the Association shall be ten dollars.
7. The Constitution and By-Laws may be amended by a vote of the Association subsequent to that at which such amendment was proposed, by vote of three-fourths of the members present.

The Secretary then read changes in the Constitution and By-Laws recommended by the Council in order to harmonize current practices with these documents, as follows:

Amend Article II of the *Constitution* to replace the words "Council of Seven, who shall" by the words "Council, which shall."

Replace Article 2 of the *By-Laws* with a new Article 2 to read: "The Council shall consist of seven Members elected by the Association, and the Secretary and Treasurer *ex officio*. The Members shall be elected for terms of seven years each, one Member to be elected annually, and shall not be eligible for immediate reelection."

Delete Article 3.

Change Article 4 to Article 3 and Article 5 to Article 4.

Replace Article 6 with new Article 5, to read: "The annual dues shall be determined by the Council."

Change Article 7 to Article 6.

The Secretary reported that through an oversight the action of the Council last year in reference to the American Board of Pathology had not been made public. Invitations from the American Society of Clinical Pathologists and the Advisory Committee on Medical Specialties had been discussed at two meetings of the Council. During recent years the Council has more and more definitely considered that the function of the Association shall be, as defined in Article 2 of the Constitution, "The advancement of the knowledge of disease," and has been reluctant to embark upon projects that do not directly bear upon this purpose. For this reason the invitations of these two bodies were declined, but at the same time the Council expressed sympathy with the objectives of the American Board of Pathology.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

VARIABILITY OF DAILY TUBERCULIN REACTIONS. John Howe (by invitation), Chicago, Ill.

Abstract. Daily intracutaneous tuberculin reactions, using simultaneous doses of 2 different dilutions of tuberculin PPD, were performed on a series of 4 patients with clinical tuberculosis and 2 control subjects, over periods ranging from 1 to 4 months. The average diameter of the reaction in millimeters was read at 24 hours, and this value was multiplied by the negative logarithm of the dose of tuberculin in millimeters to obtain the Von Gröer product. These products were then plotted on a graph, of which the baseline was the date of injection, to obtain the curves of allergy for each subject studied.

These tuberculin curves show marked fluctuations in the reaction to the same dose of tuberculin, which may be daily or over a period of several days. These fluctuations are present in varying degree in all the subjects studied. The range of fluctuation is from 50 per cent to 100 per cent of the maximal reaction in the patients, and from 55 per cent to 67 per cent in the control subjects.

There is a definite correlation between the state of the peripheral vessels, as measured by daily blood pressure readings under basal conditions, and the fluctuations in the tuberculin reaction. In general, the periods of increased tuberculin reactions are periods of vasodilatation following a period of vasoconstriction, as shown by a falling diastolic pressure following a pressor episode. The correlation between increased tuberculin reactions and falling diastolic pressure ranges from 0.77 to 1.00 in the subjects studied.

Discussion

(Dr. Max B. Lurie, Philadelphia.) I should like to know whether or not the author has found any evidence for desensitization as a result of the repeated administration of tuberculin. In guinea pigs it is frequently seen that if tuberculin is administered at very short intervals desensitization of the animal occurs, and sometimes the reverse happens, especially if the intervals are longer in duration.

(Dr. Howe.) With these small and moderate doses, which I have used, I have found no evidence of desensitization. As you can see from the curves presented, the reactions at the end of the period of observation were comparable with those at the beginning. And neither have I found any evidence for sensitization to tuberculin in these subjects due to the repeated doses.

(Dr. Esmond R. Long, Philadelphia.) I think Dr. Howe has demonstrated beautifully a relationship, a real correlation, with the diastolic pressure. It is interesting that he was not able to make any clear-cut correlation with the clinical course of the disease. Attempts have been made again and again to show such a correlation, but it cannot be worked out. Recently a group of

us followed 116 patients in the wards of the Philadelphia General Hospital over a period of a year with tuberculin tests. We recognized that there were daily variations, and we also considered no variation significant unless it exceeded 50 per cent of the original size of the reaction. We, like Dr. Howe, used 2 tests, a very weak dose and an intermediate one; we did not bother with the standard strong one. But the only correlation we were able to work out was with what might have been desensitization. About 25 pleural effusions developed in the course of the year and this complication usually depressed the intensity of the allergy. Like Dr. Howe, we saw an instance of depression of allergy following sudden pulmonary consolidation due to tuberculosis. It was noteworthy that patients who were improving and patients who were getting worse tended, as the months went by, to fall lower in their intensity of response to tuberculin.

ATTRACTION OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES BY TUBERCLE PROTEIN. William B. Wartman (by invitation), Chicago, Ill.

Abstract. In these experiments a study has been made of the chemotactic properties of one of the protein fractions of tuberculin (Seibert's water-soluble tuberculin protein b No. 50). In the undissolved state no attraction for leukocytes could be demonstrated. The protein was then brought into solution and adsorbed on kaolin and on charcoal. In this state strong positive chemotropism was exhibited by the protein in contrast with the weak chemotactic properties of the pure adsorbing agents. By statistical methods its was possible to show that the difference was a significant one. Both kaolin and charcoal were used in order to eliminate the adsorbing agent as a source of attraction.

FURTHER STUDIES ON THE MECHANISM OF IMMUNITY IN TUBERCULOSIS. Max B. Lurie, Philadelphia, Pa.

Abstract. A tuberculous rabbit or guinea pig mobilizes the mononuclear phagocytes at the site of inflammation more rapidly than a normal animal, not only in response to reinfection with the tubercle bacillus, but also when a non-specific irritant such as aleuronat is introduced into the pleural cavity. In the rabbit there is no constant correlation between the pH of the exudate and its leukocytic formula, as was also observed by Menkin. There is no evidence of greater seepage of blood proteins into an area of inflammation in a tuberculous as compared with that of a normal rabbit. Nor do antibodies circulating in the blood of rabbits accumulate in the inflamed area to a greater extent in a tuberculous than in that of a normal rabbit.

In the guinea pig there is a definite correlation between the pH of the exudate and its leukocytic formula. The tuberculous guinea pig responds to aleuronat by an exudate which is of lower pH and contains a larger percentage of mononuclear phagocytes than that of a normal animal. Blood proteins and circulating antibodies seep into an area of inflammation of a sensitized guinea pig in higher concentration than in that of a normal animal. Corresponding with these apparent differences in the vulnerability of the blood vessels of tuberculous animals of these two species are differences in their capacity to fix tubercle bacilli of reinfection.

In the rabbit, with large doses, the excessive flow of lymph from the site

of reinfection overcomes the immobilizing factors of the immune animal and accelerates the spread of the bacilli. In the guinea pig, even with large doses of reinfection, the inflammation is of such a character that the flow of lymph is retarded, and the localization of the bacilli is effective.

The extracellular factor inhibiting the growth of tubercle bacilli *in vivo* in the immune animal previously demonstrated by the use of the agar focus cannot be duplicated *in vitro* with the body fluids of the immune animal. Densely woven silk bags were impregnated with a concentration of collodion which prevented the entrance of cells, but admitted the body proteins. If such bags containing tubercle bacilli are placed within the peritoneal cavity of normal animals the bacilli grow unhindered in the bag, while in the tuberculous animal their growth is inhibited. Within these bags the bacilli grow in agglutinated masses in the immune animal; in the normal animal they are more often dispersed. There is no correlation between the thickness of the capsule formed about the bag and the growth of the bacilli within it. Whether this indicates a specific bacteriostatic property of the body fluids of the tuberculous animal or whether this results from physical and chemical conditions which are brought into play differently in the tuberculous than in the normal animal cannot be stated at present. In any event, there is no reason to suppose that these extracellular factors do not play a role in the suppression of growth of tubercle bacilli in the disease itself.

Discussion

(Dr. Valy Menkin, Boston.) I am very much interested in Dr. Lurie's paper and particularly in regard to his findings in guinea pigs which seem to be similar to what we had found previously in dogs. I was especially interested in his findings in the rabbit, for we had found exactly the same type of response, namely, that there was no strict correlation between the pH of the exudate and the cytological picture. This is contrary to the findings in the dog and in the guinea pig. We have been studying disturbances in the carbohydrate metabolism and the data that are accumulating seem to show that there may be a correlation between the degree of glycolysis and the cytological picture in the rabbit. It is to be remembered that the enzymatic properties of the leukocytes in the rabbit are somewhat different from those in the dog. Dr. Opie pointed that out a number of years ago. The rabbit evidently shows an exceptional type of behavior which ought to be thoroughly studied. It is quite possible that the disturbance in carbohydrate metabolism of that animal may yield intermediary carbohydrate products which may be of importance in determining, at a given time, the predominating type of cell in the rabbit exudate.

(Dr. Paul R. Cannon, Chicago.) I should like to ask Dr. Lurie how he determined the bacterial content in the silk sac, and if he thinks that the reduction in numbers is due to an ablastic antibody, such as Taliaferro has shown for trypanosomes.

(Dr. A. L. Joyner, New York City.) I should like to ask if Dr. Lurie measured the pH of the collodion sac.

(Dr. Lurie.) In regard to Dr. Menkin's remarks, I wish to state that despite the fact that we could not regularly demonstrate pH differences in the exudate of the tuberculous animal as compared with that of the normal animal, there

is much to show that there is a great deal more injury produced on the cells in the exudate of the tuberculous animal as compared with that of the normal animal. Whether this pH inconstancy is accounted for by the neutralizing and buffering effect of the blood proteins I am not ready to say.

In reply to Dr. Cannon, the number of bacilli present in the bag of the tuberculous animal as compared with that of the normal animal was determined by taking a weighed amount of agar from each bag. I did not have a chance to say that the tubercle bacilli were placed inside the bag in molten agar. A weighed amount of the agar inoculum as well as that within the bags was ground as thoroughly as possible; microscopic examination showed the bacilli were completely isolated after grinding. I determined the number of living tubercle bacilli by the number of colonies cultured. Microscopic examination of the agar in these bags showed that fewer colonies developed within the bag placed in the peritoneal cavity of a tuberculous animal, and secondly, and I think that is even more important, in a normal animal the bacilli grow both in colonies and in dispersed form, but in the tuberculous animal they more often grow in an agglutinated manner. Whether this inhibition of growth is due to a specific bacteriostatic property of the serum or body fluids, or whether it is due to a physicochemical change or reaction that takes place differently in a normal as compared with a tuberculous animal I am not ready to say.

The pH of the agar within the silk bag is distinctly lower in the tuberculous animal than in the normal animal, in the small number of observations thus far made. If, as sometimes happens, the growth of the bacilli is significant in the immune animal, then the pH of the agar in that bag is not lower than that in the normal animal.

THE DEVELOPMENT OF TUBERCULOSIS IN NON-ALLERGIC GUINEA PIGS. C. E. Woodruff and H. S. Willis, Northville, Mich.

Abstract. In the attempt to throw added light on the question of the relation between allergy and immunity in tuberculosis, the development of that disease has been studied in more than 500 normal, allergic and desensitized guinea pigs. Some groups of animals, rendered allergic by R_1 infection, were desensitized by means of subcutaneous doses of tuberculin gradually increased until 1 cc. of undiluted OT was being given daily. Other groups, infected from the outset with virulent organisms, were kept from developing allergy by means of daily 1 cc. doses of tuberculin. These latter animals fared the worst of any group, showing a 100 per cent mortality, usually by the end of the 3rd month. In all of our mortality studies some of the allergic animals continued alive long after all the desensitized and unsensitized pigs were dead.

Our experiments indicate that the development of tuberculosis is modified to a marked degree in guinea pigs desensitized, or kept non-allergic, by means of large parenteral doses of tuberculin. In these animals the liver and spleen are spared to a large degree, but at the expense of the lungs. There is little or no tubercle formation, the lungs becoming the seat of a fulminating tuberculous pneumonia. Smears from such lungs show innumerable tubercle bacilli. Experimental studies reported in the literature, in which all the animals have been sacrificed before the end of the 3rd month, may fail to give a proper comprehension of this extensive development of pulmonary disease in the desensitized guinea pigs.

PATHOLOGICAL EVIDENCE OF AXONAL AND TRANS-SYNAPTIC PROGRESSION OF VESICULAR STOMATITIS AND EASTERN EQUINE ENCEPHALOMYELITIS VIRUSES. Albert B. Sabin (by invitation) and Peter K. Olitsky, New York City.

Abstract. Previous studies on the development with age of localized barriers to the progression of vesicular stomatitis (VS) virus in the peripheral and central nervous systems suggested that the movement of peripherally injected virus occurred in a closed system not only along the axons but probably across the synapses as well. Hurst's studies on Eastern equine encephalomyelitis (EEE) virus led him to state that its movement was not along the axons because it could not spread along peripheral spinal nerves such as the sciatic.

An attempt was made to determine from the distribution of lesions the pathways pursued by the VS and EEE viruses within the CNS, and whether the inability to spread centripetally along certain peripheral nerves implied that axonal progression in general was impossible. Mice were given one or the other virus (1) intracerebrally, (2) intranasally, (3) into the vitreous of 1 eye or (4) into the muscles of 1 hind leg. The whole CNS was sectioned semiserially and the location of lesions noted. After intracerebral injection the lesions were chiefly periventricular, indicating cerebrospinal fluid dissemination, while after the peripheral inoculations the distribution of lesions was wholly different and depended on the central connections of the nerve along which invasion occurred, suggesting primary axonal and trans-synaptic progression. After nasal instillation of either virus in young mice the destruction of neurones in the olfactory pathway from the primary ones in the nasal mucosa through a consecutive chain of 3 to 6 others within the CNS was readily demonstrable. From the vitreous both viruses followed the optic nerve pathway and the constant lesions were (1) in the neurones of the retina, and (2) in those of the contralateral superior colliculus. When the right eye was injected, only the left superior colliculus was necrotic, and only the right one when the left eye was inoculated. The two viruses behaved differently after intramuscular injection. In young mice VS virus passed up the peripheral spinal nerves and produced lesions in the cord. With EEE virus this occurred in only about 5 per cent of the mice, while in the others it was eliminated from the blood onto the olfactory mucosa and the CNS lesions were then the same as after nasal instillation. That EEE virus in the blood invades the CNS by the olfactory pathway was proved by the fact that preliminary treatment of the nasal mucosa with tannic acid, which blocked that pathway, resulted in survival without CNS signs.

THE NATURE AND RATE OF CENTRIPETAL PROGRESSION OF CERTAIN NEUROTROPIC VIRUSES ALONG PERIPHERAL NERVES. Albert B. Sabin (by invitation), New York City.

Abstract. The mechanism whereby a neurotropic virus introduced into a muscle invades the CNS along a peripheral nerve, and the relation of alleged differences in the rate of centripetal progression to variations in incubation periods of different viruses were studied. The pantropic pseudorabies and B viruses were employed. (The incubation period after intramuscular injection of rabbits is in the former 50 to 60 hours, in the latter 6 to 7 days.) A constant

amount of virus was injected into leg muscles supplied chiefly by the sciatic nerve and a number of segments of the nerve were tested at varying intervals. By this method no stepwise progression of virus up the nerve could be demonstrated. Instead, with B virus, there was a period of 48 to 72 hours when, with abundant virus in the muscle, none was detectable in any part of the nerve or cord; between 72 and 96 hours the caudal and cephalad halves of the nerve and the cord became positive together and remained positive thereafter. The total amount of virus in the nerve at 96 hours was so small that none could be detected in 1 cm. pieces removed from the middle of either the caudal or cephalad halves, the remainder of which was positive. This type of experiment suggested that primary spread was not by multiplication, rendering the detection of virus at 96 hours possible only in large neural segments. That multiplication occurs later is evident from the increase of virus in the entire nerve, making its detection easy in the small pieces (at onset of paralyses on the 7th day).

The following experiment indicates that intramuscularly injected virus progresses centripetally only along the axon: 1 cm. of the sciatic nerve was excised midway in its course in the thigh; this leaves essentially intact the other neural structures but cuts the axon from its cell body. The excision was made at 48 hours after injection and rabbits were sacrificed at 72 and 96 hours, 8, 9 and 11 days. In none of ten animals was virus detected in the caudal half of the nerve, despite the fact that it was attached to the muscle containing to the end abundant virus, and this result was shown not to be due to the development of inhibitory or antiviral substances in the cut nerve. It was clear that when the axons were severed the spread of virus along them was completely inhibited. At the same time it became evident that no centripetal progression occurred along the other structures of the nerve attached to the muscle.

Studies with pseudorabies virus revealed that none could be detected in the nerve during the first 24 hours; it became demonstrable at 30 to 40 hours in the entire nerve and the cord. It thus appeared that the rate of progression along the nerve did not by itself determine the incubation period. The latter seemed to depend rather on (1) the initial latent period which probably represents the interval before the virus begins to spread along the nerve (B virus 48 to 72 hours; pseudorabies 24 to 30 hours), and (2) the time between invasion of the cord and appearance of sufficient cellular damage to give rise to clinical signs (B virus 3 to 4 days; pseudorabies 12 to 24 hours).

Discussion

(Dr. Arthur W. Wright, Albany.) I should like to ask Dr. Sabin if, while carrying out this work on axonal transmission of viruses, any studies were made of the morphological changes in the affected peripheral nerves. I am wondering whether or not he was able to demonstrate any definite structural changes in the axis cylinders of the nerves that had transmitted virus.

(Dr. Sabin.) At the onset of paralysis, *i.e.*, 3 to 4 days after B virus is first detectable in the peripheral nerve conveying it, widespread lesions are seen in almost all the structures of the nerve, including the axis cylinders. At this time, however, there are also lesions in the neurones of the spinal ganglia and cord, of which the axons are but the protoplasmic extensions. Thus far,

therefore, pathological studies alone have failed to throw light on the course of events. Since the first visible change in the neurone is in the nucleus one must consider the possibility that the virus may have to travel up the long axis cylinders to the nucleus before any increase or multiplication can occur.

HAPTEN CONJUGATION WITHOUT PROTEIN DENATURIZATION. Sol Roy Rosenthal, Chicago, Ill.

Abstract. While working with phenolphthalein it was noted that this drug administered enterally or parenterally soon appeared in the blood in free and combined forms. For the parenteral route the highest concentration of combined phenolphthalein in the blood was found after 1 to 1½ hours. (Combined phenolphthalein is determined by heating the phenolphthalein serum in a water-bath for 2 hours with concentrated hydrochloric acid and extracting same with ether. The amount of phenolphthalein in the extract is determined in the usual colorimetric way.) The substance to which the phenolphthalein becomes combined is not known, but it is suspected of being protein in nature. This combined hapten-serum was used as an antigen, and injected into a different species of animals than that in which the combination was accomplished. For haptens, phenolphthalein, old tuberculin, acetone soluble and acetone insoluble-methyl alcohol soluble fractions of the tubercle bacillus were used. Precipitin ring tests and skin tests were made, using for antigen the original haptens and the serum of a third species of animal in which the hapten had been injected. Thus, for example, a hapten was first injected into a rabbit intramuscularly; blood was withdrawn after 1 to 1½ hours, and this serum was injected into a guinea pig by any of the parenteral routes. This was repeated every 5 to 6 days for 8 injections. One week after the last injection blood was drawn to be used in the precipitin tests. For antigen use in the precipitin and skin tests the hapten was injected as originally but into a cat or rat, and blood was drawn after 1 to 1½ hours. Control serological and intradermal tests were done using the serum of the same cat or rat before injecting the hapten.

The results indicate that the precipitin tests were not always constant, the best tests being obtained with the extracts of the tubercle bacillus and tuberculin when the combined antigen was injected intravenously. These were positive in dilution up to 1:100,000 of the antigen, and could be completely effaced by adsorption, using the hapten only.

Skin tests gave more constant results, the most striking being with tuberculin, phenolphthalein or the combined serum-hapten antigen. The skin reactions after intracutaneous injection were manifest by induration and redness, at times covering areas 50 by 50 mm. In the case of tuberculin these skin manifestations were already manifest after 1 hour, being most pronounced after 6 to 24 hours, but persisting for 48 to 72 hours, many showing central areas of necrosis. In the case of phenolphthalein the reactions were manifest after 1 to 2 hours and persisted for 48 to 72 hours. Hemorrhages in the lesion were frequent. Control animals yielded little to no reactions. The tuberculin animals after 5 months still give positive skin tests.

This report is a preliminary one. There are many complex features that need solving.

Discussion

(Dr. Augustus B. Wadsworth, Albany.) I should like to ask if any precipitation occurs when the acetone-insoluble extract is added to the normal rabbit serum — the rabbit that was not immunized.

(Dr. Rosenthal.) As indicated in the tables projected, normal serum in some instances caused precipitation with the hapten-serum combination, but not as strong as in the immunized animals. No precipitation resulted when the hapten alone was added to normal or immune serum.

SEROLOGICAL STUDIES OF THE REPTILIA. III. STUDIES OF HEMAGGLUTININS IN SNAKE SERUM FOR HUMAN ERYTHROCYTES. Glenn C. Bond (by invitation), Lawrence, Kan.

Abstract. In a study of 112 samples of snake serum representing 10 genera and 18 species it was found that 31.2 per cent did not agglutinate human erythrocytes, 19.6 per cent agglutinated types A, B, and AB human erythrocytes, 33 per cent agglutinated all 4 types of human erythrocytes, 1.8 per cent agglutinated A and AB erythrocytes, and 14.4 per cent gave irregular results.

The agglutinins for Types A, B, and AB cells can be specifically absorbed with the homologous cells. The agglutinins for type O cells are absorbed out by any type of human cells. Appropriate snake serum absorbed with human A cells and serum absorbed with human B cells can be used to determine human blood types.

The stability of these agglutinins is comparable to those in human serum.

ISOLATION AND CHEMICAL ANALYSIS OF THE ERYTHROGENIC TOXIN OF *Streptococcus scarlatinae* (NY 5 STRAIN). A. H. Stock (by invitation), Pittsburgh, Pa.

Abstract. From a sterile filtrate of NY 5 strain of the hemolytic streptococcus, the toxin was precipitated by two volumes of ethyl alcohol at 0°. The nucleo-protein was removed from the water-soluble part of this precipitate by the addition of acetic acid to pH 4. After neutralization, alcohol precipitation and solution were repeated twice. The precipitate was dialyzed in a No. 600 cellophane bag and then dried. By means of this procedure 0.3 gm. of a viscid substance, drying in thin plates, was obtained from each liter of filtrate. The product is a hydroscopic nitrogenous polysaccharide which contains a minimum of 1.5 per cent ash and 7 per cent nitrogen. It gave no reduction of Benedict's solution, no amino nitrogen with van Slyke procedure, low phosphorus, no halogen or sulphur, negative ninhydrin, faintly positive biuret test, and strongly positive Molisch. After acid hydrolysis, 60 per cent reduction (calculated as glucose) was obtained. The reducing material was N-acetyl glucosamine and galactose. The former was identified by isolation of glucosamine hydrochloride and conversion into the anisaldehyde derivative, and determined quantitatively as 37.5 per cent of N-acetyl glucosamine. Galactose was identified by oxidation to mucic acid. An additional 2 per cent acetyl was present. With the naphthoresorcinol test for uronic acids a dark reddish blue benzol extract was obtained. This extract showed no spectrum. A maximum of 4 to 5 per cent uronic acid was determined by measuring CO_2 from HCl hydro-

lysis. The remaining nitrogen-containing fraction, amounting to 40 per cent of the material, could not be identified owing to interference by glucosamine. One mg. of the toxin (approximately 3 cc. of original broth) gave 75,000 STD. Since 3 cc. of the original broth gave approximately 90,000 STD, it is believed that the bulk of the toxin was removed. The toxin has a proved high immunizing capacity in susceptible individuals.

From Parke Davis peptone, or from the uninoculated medium, a polysaccharide similar in composition to that obtained from the streptococcic filtrate has been isolated. Apparently this fraction is identical with the blood group A substance obtained from pepsin by Schiff and by Landsteiner. Its presence in the Parke Davis peptone (and probably in other peptones as well) is ascribed to the use of pepsin in the manufacture of peptone. Attempts to fraction further the isolated toxin have to date not been successful. Whether the polysaccharide from peptone is an integral part of the precipitated toxin requires further investigation.

Discussion

(Dr. M. L. Menten, Pittsburgh.) Preliminary tests made in our laboratories on a considerable number of peptones showed that all of them gave a very marked Molisch reaction. The question arises as to the relationship of the polysaccharide contained in the uninoculated broth to that of the toxin. In our opinion, one cannot conclude that because the same chemical procedure gives a precipitate from the streptococcic filtrate but fails to give a precipitate from the uninoculated medium, the latter does not contain the constituents of the bacterial polysaccharide. Before one can draw definite conclusions regarding the polysaccharide of the bacterial products found during growth in the culture medium, it is necessary to go back to a critical study of the peptones.

(Dr. Augustus B. Wadsworth, Albany.) Does the antitoxin which is obtained by inoculation of animals with your purified toxin act in the same manner with other toxins as the antitoxin which is produced with your original crude toxin? Streptococcus toxins exhibit peculiar specificities: two toxins may be very closely related but the antitoxin of one will neutralize both, and the antitoxin of the other only the homologous toxin. The antitoxin of the NY 5 strain is broadly valent, whereas some closely related toxins produce antitoxins of much narrower valency.

(Dr. Stock.) We have not used the isolated toxin as yet for immunization to produce antitoxic serums. It has been employed only for immunizing susceptible individuals, and it does immunize them to a negative skin reaction. Our serological tests have been confined to Dick skin tests.

CHEMICAL AND SEROLOGICAL DIFFERENTIATION BETWEEN THE PROTEINS OF DIPHTHERIA TOXIN AND DIPHTHERIA BACILLUS. Monroe D. Eaton (by invitation), St. Louis, Mo.

Abstract. The purpose of this work was to determine what proportion of the purified toxic protein, which possesses all of the properties of diphtheria toxin, consists of bacterial protein. The bacterial precipitinogens were detected and measured by precipitin tests with a serum prepared by immunizing a

rabbit with washed diphtheria bacilli. This serum contained very little antitoxin.

The carbohydrate precipitinogens in crude diphtheria toxin are eliminated during the purification of the toxin. One of the bacterial proteins is easily precipitated out of the preparations by one-third saturated ammonium sulphate. A second protein precipitinogen may be partially separated from the toxin by ammonium sulphate and acid fractionation. This bacterial protein, when isolated, gives with the antibacterial serum a precipitin ring at 1:300,000, a dilution of protein 50 to 100 times as great as the corresponding titer dilution of toxic protein against the same serum. Antibacterial serum absorbed with the non-toxic protein precipitinogen gives positive precipitin tests with crude toxin, but not precipitate with the purified toxic protein.

These measurements by the precipitin test indicate that bacterial protein constitutes less than 2 per cent of the total protein in the most highly purified preparations. No protein was present in the culture medium used for production of the toxin. The readily soluble protein, which appears to be diphtheria toxin, is both chemically and serologically distinct from the somatic proteins of the diphtheria bacillus.

A Quantitative Study of the Ramon Diphtheria Flocculation Reaction. Alwin M. Pappenheimer, Jr., and Elliott S. Robinson (by invitation), Jamaica Plain, Mass.

Abstract. A quantitative study of the diphtheria flocculation reaction by determination of the nitrogen specifically flocculated by the combination of antitoxin and toxin is described. The results of this study have made possible the following calculations and deductions:

1. Diphtheria toxin and antitoxin unite in more than one proportion. The Danysz's phenomenon may be explained in these terms and the magnitude of its effect calculated from the results obtained.

2. The limits of the equivalence zone over which toxin is neutralized by antitoxin have been determined.

3. The amount of nitrogen per Lf unit of diphtheria toxin, the nitrogen per unit of antitoxin and the ratio of antitoxin nitrogen to toxin nitrogen throughout the equivalence zone have been calculated.

4. The analogies and differences between the flocculation and precipitin reactions have been discussed.

5. An absolute method has been furnished for determination of the potency of toxin and antitoxin preparations, even though the strength of neither one is known.

Discussion

(Dr. A. M. Pappenheimer, Jr., Boston.) I wish to point out that we have found it necessary to postulate the formation of these soluble intermediates in the union of diphtheria toxin antitoxin in order to explain the Danysz's phenomenon, which is manifest even though no precipitation occurs. The combination of antigen and antibody in multiple proportions completely explains the Danysz's effect only if precipitation occurs, as with the bacterial polysaccharides. Using our results it is possible to calculate and forecast the magnitude of the effect.

TUBULAR DISEASE OF THE KIDNEYS. E. T. Bell, Minneapolis, Minn.

Abstract. Tubular disease of the kidneys is a much less frequent cause of uremia than glomerular disease. Eight cases have been studied in which there is satisfactory evidence that uremia was due entirely to injury of the tubules. Six of these were instances of mercuric chloride poisoning, and 2 were due to toxemia. In the latter 2 cases there was a severe hydropic degeneration of all the convoluted tubules.

The symptoms referable to tubular disease are oliguria or anuria and azotemia. Albuminuria and edema are due to glomerular disease.

In the uremia following transfusion with incompatible blood, anuria is due largely to obstruction of the tubules by casts of hemoglobin, but tubular injury may be a contributory factor. In 11 cases of acute uremia with marked hematuria, the renal insufficiency seemed to be due to obstruction of the tubules with blood.

In 8 cases of acute uremia there was marked enlargement of the kidneys and some tubular injury, especially dilatation, but the injury seemed insufficient to have caused uremia and one must suppose that extrarenal influences played a role in causing urinary suppression.

In 7 cases of acute uremia the kidneys were entirely normal microscopically, and urinary suppression must be attributed entirely to extrarenal influences.

The most important factors in the etiology of extrarenal uremia are dehydration and increased destruction of tissue protein. A decrease of blood chloride is apparently not a cause of azotemia.

Discussion

(Dr. Paul Klemperer, New York City.) I should like to ask Dr. Bell about the extrarenal uremia. You have probably seen cases of so-called hepatorenal syndrome which the clinicians are so much interested in, and I wonder what your findings are. In my own experience I could not find any definite change in the kidney to account for the uremia, and I wonder if Dr. Bell's experience has been the same.

(Dr. Virgil H. Moon, Philadelphia.) I am interested in two of the types of renal disease that Dr. Bell has shown: one, the hemorrhagic type, which was associated with septicemia; the other, similar in its hemorrhagic features, in which the condition followed transfusion. I wonder whether Dr. Bell believes that the hemorrhagic features may have originated from a primary injury to the capillary endothelium of the kidney. The reason for making the suggestion is that I have seen lesions similar to these following both clinical shock and experimental shock produced by various means in animals. In those instances the hemorrhagic features were apparently due to direct injury to the capillary walls by whatever agent was responsible for the circulatory deficiency.

(Dr. Bell.) In reply to Dr. Klemperer's question, I have not actually had an example of the hepatorenal syndrome he mentions, but from published reports of these cases I judge they are examples of dehydration, as in intestinal obstruction. This is called hypochloremic uremia, but we know that it is the lack of water and not low blood chloride that causes azotemia.

In the hemorrhagic type of glomerulonephritis which I mentioned I think the injury to the glomerular capillaries is the primary disturbance because

the tubules are usually filled with blood and only occasionally with hemoglobin. But in the transfusion kidney the situation is different. Hemoglobin is formed in the blood stream, passes through the glomeruli and is precipitated in the tubules, obstructing them when the urine is acid.

THE PATHOGENESIS OF CORTICAL NECROSIS OF THE KIDNEY IN RABBITS FOLLOWING THE INJECTION OF STAPHYLOCOCCUS TOXIN. John H. Glynn (by invitation), Montreal, Canada.

Abstract. The wide individual variations in susceptibility of animals to staphylococcus toxins can be controlled by attention to two factors: first, the recognition that a considerable proportion of normal animals possess so-called "natural" antitoxin; and second, the measurement of toxin in terms of some unit of activity. Under such conditions reproducible results are obtained. The present study was directed toward an answer to the question of whether kidney necrosis is due to a direct action of staphylococcus toxin on renal epithelium or secondary to vascular damage. Evidence based on mitochondrial changes preceding hemorrhage supports the former viewpoint, although vascular damage quickly follows and the final picture is believed to be the resultant of both factors. Hemorrhage begins in the glomeruli with extreme dilatation and eventual rupture of capillary loops.

Discussion

(Dr. Joseph Tannenberg, Albany.) By chance I made some time ago an observation which greatly supports the view that at least certain poisonous substances injected intravenously may act directly on the epithelium of the convoluted tubules. Rabbits were injected with zinc sulphate intravenously and sacrificed 48 hours later. Within that time the drug in the employed doses produced marked degenerative, necrobiotic changes in the epithelium of the convoluted tubules uniformly throughout the cortex. In one instance, however, a rabbit so injected happened to be afflicted with a spontaneous localized chronic nephritis which had produced many small foci scattered over the cortex. The tubules within these chronically inflamed areas were greatly distended and exhibited a low regenerated epithelium similar to that lining regenerated tubular areas encountered in the course of chronic glomerular nephritis in man (islands of Stoerk). In our case it was striking that these regenerated epithelial cells proved to be entirely free of degenerative changes produced by the zinc sulphate, as if they were specifically protected. This protection was obviously due to the fact that they failed to absorb and concentrate zinc sulphate from the primary glomerular urine, as the normal epithelium of the convoluted tubules did to such a degree that it became necrotic. Under the assumption that the poisonous drug had primarily acted on the blood vessels and by this way influenced the tubules, the protection and preservation of the degenerated tubules would be difficult to explain.

(Dr. A. L. Joyner, New York City.) Some years ago Dr. R. H. Rigdon and I injected toxin in rabbits and reported later on a nephritis we observed, and it was mentioned that we had a good deal of difficulty in getting animals with consistent results, but we observed these early changes in the tubules, and it was entirely on the basis of these that we drew our conclusions. We observed early changes in the mitochondria and cloudy swelling of the tubules at the

same time. We did not lose sight of the fact, however, that when we injected toxin we also got extraordinary changes in the blood vessels, and I disagree with the Doctor that the nephritis is the most common change that takes place in the animal. We saw nephritis only in 25 per cent of the animals. We frequently had sudden death out of some 250 rabbits injected. Sometimes death occurred in 5 to 6 hours, or in 24 hours, and we never saw nephritis. We frequently saw tremendous changes in the blood vessels, and at that time the nephritis mechanism we felt started in the tubules and was a quantitative thing depending on how much toxin was injected and how long the animal survived after the original insult, but we did not lose sight of the fact that the tremendous changes in the blood vessels were the commonest things.

(Dr. Glynn.) In answer to Dr. Joyner, I quite agree with him that if large doses of toxin are used the easiest thing to produce is acute death, and of course if the animals die within a minute, or as early as 5 minutes, there is not much histological damage that can be demonstrated by any staining method I know of. The point I tried to bring out is this — that there are changes that occur in the tubules at an early stage if the animal lives. But the animals must be selected on a basis of preliminary titrations for natural antitoxin and the activity of the toxin must be measured at the time of its use. All these animals were selected on this basis and were given an injection of toxin which was less than a lethal dose. The changes that occur in the tubules can then be shown to be due to the direct action of the toxin and are not secondary to the vascular damage. The hemorrhage is a later phenomenon. These early changes cannot be detected with hematoxylin and eosin stains because a kidney that shows a great deal of damage to the mitochondria by a mitochondrial staining method may by routine methods show no change at all.

EXPERIMENTAL EMBOLIC GLOMERULONEPHRITIS PRODUCED BY HUMAN FAT, FATTY ACIDS AND CALCIUM SOAPS. C. S. Hagerty (by invitation), University, Alabama.

Abstract. Lesions similar to those seen in glomerulonephritis in man can be produced in kidneys by the injection of irritants in the form of emulsified human fat, human fat containing soaps, oleic acid and liquid petrolatum into the renal arteries of dogs and rabbits.

Microscopically the fat emulsions injected into the renal arteries affected the kidneys in two ways. The lipins stimulated tissue reactions in the glomeruli, the nature of which depended on the chemical composition of the material injected. The obstruction in the capillary tufts of the glomerulus by the droplets or reactive tissues or both caused atrophy, necrosis, or fatty changes of the cells lining the tubule portion and a subsequent growth of connective tissue about this structure.

The glomerular lesions are of two varieties and depend on the chemical composition of the irritant. Mild irritants cause tissue reactions characterized by endothelial proliferation with a minimum production of collagenous material. Strong irritants produce glomerular reactions in which there is endothelial swelling and marked production of collagenous material. Moderate irritants cause both endothelial proliferation and collagenous production.

Discussion

(Dr. E. T. Bell, Minneapolis.) Dr. Hagerty, don't you think that the collagen which you found in the glomeruli is derived from capillary basement membranes? In all the embolic forms of nephritis I have studied the occurrence of collagenous fibers in the glomeruli is due to splitting of the capillary basement membranes. There are no fibroblasts in the glomeruli.

(Dr. Frederic Parker, Jr., Boston.) How did you identify those cells as epithelial cells? In my experience we often see many mononuclear cells, and it is very difficult to tell exactly the type of cell.

(Dr. Hagerty.) In Mollendorf's Handbook of Microscopic Anatomy there is an illustration which indicates that there are fibroblasts in the glomerulus. In their reaction to an irritant these cells might produce collagen. I think that many of the photomicrographs which you saw indicate what Dr. Bell has said, that the fibers seem likely to split off from the basement membrane because they are most numerous in that region. I cannot be certain of the exact nature of the blue staining material which occurs between the endothelial cells.

In answer to Dr. Parker's question, it is a difficult thing to decide whether these cells are monocytes or endothelial cells. However, their appearance and the association of these cells with fibers which lead to a permanent scar indicate to me that the reaction about the fat droplets is a proliferative one.

PARATHYROID HYPERPLASIA IN CHRONIC RENAL INSUFFICIENCY. Benjamin Castleman and Tracy B. Mallory, Boston, Mass.

Abstract. Another case of "primary" hyperparathyroidism characterized by diffuse hyperplasia of the parathyroid glands of the wasserhelle type is reported. The histological findings in this case have been used to emphasize the contrasting character of the "secondary" hyperplasia which is described in detail on the basis of 27 cases of chronic renal insufficiency of varying grades. Whereas in the primary hyperplasias a uniform direction of differentiation of all cells to the large water-clear type is the invariable finding, in the secondary hyperplasias such uniformity is lacking. Here the glands are composed almost completely of normal sized chief cells, although a few small water-clear cells are occasionally present. The oxyphil cells are always greatly increased in number. The glands show varying degrees of gross enlargement and even when the enlargement is limited to a single gland, microscopic examination has not failed in any instance to show evident hyperplasia in the other glands as well. The criteria for the diagnosis of secondary hyperplasia are described. Comparison of cases of chronic renal insufficiency with and without bone lesions showed quantitative but not qualitative differences in the parathyroid glands, and the development of osteitis fibrosa is felt to be directly dependent on the duration of renal insufficiency. That these changes are in no way specific to renal insufficiency is shown by the fact that no qualitative differences could be recognized between the milder grades of secondary hyperplasia in nephritis and those occasionally seen in individuals without renal insufficiency, but with a variety of associated lesions varying from metastatic carcinomatosis involving bone to basophilism of the pituitary.

Discussion

(Dr. F. A. McJunkin, Chicago.) I should like to ask whether the myocardium was examined for lesions which might be associated with this condition of parathyroid change and with a disturbance of calcium and phosphate metabolism. This perhaps touches only a minor angle of the paper, but I am led to ask the question because in one of a set of experiments on nephrectomized rats it was found that myocardial lesions were present in a certain number of cases. If these bilaterally nephrectomized rats were given small to moderate doses of acid phosphate then the myocardial necroses were present in quite a large percentage of the rats, and from a hurried glance at the tables just now shown it appears that the phosphorus really was quite high in a certain number of the cases. For that reason I should like to ask if acute myocardial lesions were looked for, especially in those cases that had a very high phosphorus.

(Dr. H. Gideon Wells, Chicago.) Cases of renal rickets, so-called, from congenital malformations may produce the most spectacular parathyroid hyperplasias, and in one such case which we saw recently the myocardium showed areas of calcification necrosis quite identical with those Dr. McJunkin has produced experimentally. There is a striking similarity between the effects on the heart of phosphatemia produced by these developmental defects and the changes he produces in experimental animals.

(Dr. William Boyd, Winnipeg.) I should like to ask Dr. Mallory if he has anything to say about the possibility of a reversal of the action, with the parathyroid activity being the primary cause of the renal lesion, rather than the changes in the parathyroid being secondary. I have in mind some experiments which Dr. Bruce Chown of Winnipeg performed recently. He found that injections of parathyroid extract caused subepithelial deposits in the renal tubules, and that these deposits pushed the lining epithelium forward to such a degree that tubular blockage occurred, so that many of the animals died of renal insufficiency. It would appear that parathyroid overactivity can cause renal insufficiency, in addition to renal insufficiency causing parathyroid hyperplasia.

(Dr. Sheldon A. Jacobson, New York City.) Those who have worked with the thyroid and its disorders have in many cases long had the feeling, as is well known, that the thyroid dysfunction might not be primary in Graves' disease, but might possibly be secondary, and the various anatomical changes with function of the thyroid in diseases other than Graves' disease lend some support to this view. There is no doubt that hyperparathyroidism itself can produce a picture similar to that of von Recklinghausen's disease. Whether it is identical is a question, and there have been a few workers who have been most interested in that field who have been of the opinion that a somewhat analogous relationship exists — that the parathyroids may be involved secondarily to some unknown factor. Such observations as those reported lend some additional weight to that view.

(Dr. Mallory.) In answer to Dr. McJunkin, in all these cases we had routine sections of the myocardium and I did not notice any acute myocarditis. We did not cut many blocks, or look particularly hard for it, however.

Dr. Wells spoke of renal rickets, and that, to our mind, is exactly the same syndrome as that I have shown here, the only difference being that it occurs

in a child before the epiphyses have united rather than in an adult. It is a question whether such cases really should be regarded as rickets at all.

As Dr. Boyd pointed out, there is a theoretical possibility of a completely reversible interrelationship: overactive parathyroids can produce renal insufficiency as the result of calcium deposits in the kidneys, and a primary renal insufficiency can produce hyperplasia of the parathyroids and probably real hyperparathyroidism. In 1 case of renal rickets Schelling showed there was an increased amount of parathyroid hormone in the blood, and the bone changes in these cases are indistinguishable from those of primary hyperparathyroidism. It is possible to start at either end of the picture and wind up with the end-stage, and for that reason the possibility of distinguishing simply from the morphology of the parathyroids the two types of hyperplasia has practical significance. In some cases with an inadequate history we might have difficulty in doing it on clinical grounds.

In reply to Dr. Jacobson's question whether this disease is primary in the parathyroid or not, I can only say that certainly some of the cases are not primary. This postnephritic group is certainly not. I do not think that the diffuse hyperplasias of the water-clear type are primary either; the fact that after doing a subtotal parathyroidectomy the disease continues and the defect occurs again would point to its not being primary. We feel sure it is outside the parathyroid but have no idea of the etiology. In the group where the changes in the parathyroid glands are localized our experience at the Massachusetts General Hospital has been in every instance that removal of the localized nodule has resulted in an apparently complete cure. Most of our cases have not run longer than 2 or 3 years, but we have several cases of 5 years duration without recurrence. Therefore this group of cases we feel represents primary disease of the parathyroid organ itself.

Myocardial Hyperplasia in Cardiac Hypertrophy of Infancy. H. Edward MacMahon, Boston, Mass.

Abstract. The statement that "in myocardial hypertrophy the increase in size of the heart is due solely to an increase in size of the individual muscle fibers, and is not the result of a multiplication of these fibers" has been so frequently repeated in textbooks and in journals that it may now be considered as practically axiomatic. This is based primarily on two indisputable facts in respect to the fully mature heart: first, careful measurements of the muscle fibers of hypertrophied hearts have shown that the increase in size of hearts may be explained mathematically on the basis of an increase in size of the individual myocardial fibers; and secondly, patient search through the myocardium in cases of cardiac hypertrophy has failed to reveal any positive evidence, in the form of mitoses, of true myocardial proliferation — a fact that is strongly supported by the lack of regenerative power of heart muscle fibers following severe injury.

The purpose of this paper is not to contradict but rather to modify the above mentioned statement by pointing out that in the infant, at least until the end of the 2nd year, cardiac hypertrophy, whether primary or secondary, may be accompanied, not only by an increase in size of the individual fibers, but by an active proliferation of these as well.

In a careful examination of the myocardium of infants whose hearts were

definitely increased in weight, comprising cases of so-called primary idiopathic hypertrophy, as well as those of left ventricular hypertrophy of renal and aortic origin, it has been possible to demonstrate mitotic figures in varying stages of nuclear division. No part of the ventricular myocardium has been particularly favored, though they appear to occur more often in the outer portions of the myocardium and toward the tip of the left ventricle. It is possible that this should not be considered as an example of true hyperplasia but merely an acceleration of a normal physiological process in which the child's heart at this age is still undergoing a natural histiogenesis.

It is difficult to determine at what age the child's heart may be considered to have acquired its full complement of myocardial fibers, but the paucity of mitoses in average sized hearts of children during the first 2 and 3 months of life is so commonly recognized as to suggest that the active proliferation of muscle elements under ordinary conditions ceases quite early.

Since growth of the myocardium by the active proliferation of muscle elements may be demonstrated in the enlarged hearts of infants in the 1st and 2nd years, it is possible that during this same period myocardial regeneration may occur as well.

Discussion

(Dr. Robert A. Moore, New York City.) I should like to ask Dr. MacMahon if he has any quantitative observations similar to those made by Drs. Karsner, Saphir and Todd on the hypertrophic adult heart. We have recently studied a case of idiopathic hypertrophy of the heart in a 1 year old infant, not of the von Gierke type, in which, using these methods and counting the number of fibers in a large number of fields we could find no evidence that there was an increase in the number of muscle fibers and no increase in the number of nuclei.

(Dr. MacMahon.) In answer to Dr. Moore's question in regard to the hypertrophy of the muscle fibers in infants' hearts, I can say that one does find a true hypertrophy of the muscle fibers in hypertrophied hearts of infants. One finds this whether the hypertrophy is of the primary idiopathic type or whether it is associated with deposition of glycogen or secondary to hypertension, coarctation or renal disease. It is awfully difficult to attempt to estimate mathematically the weight of a child's heart by measuring the width of the muscle fibers alone, for these vary so in size and at times one finds hypertrophied fibers and less developed fibers lying side by side. I feel that in addition to hypertrophy that is present in these cases, the possibility of a true proliferation of heart muscle fibers should not be entirely overlooked, especially at this very early age.

THE HISTOPATHOLOGY OF EXPERIMENTALLY PRODUCED ENDOCARDITIS. Alexander Nedzel (by invitation), Chicago, Ill.

Abstract. Pitressin injections cause a rise in blood pressure by stimulating the blood vessels by peripheral action, evoking the constriction of the finer arterioles and capillaries. Periods of high blood pressure (ARS phase of Petersen) are followed by periods of low blood pressure, because with a period of spasm an anoxemia prevails in certain tissue; this causes stimulation, products of incomplete tissue metabolism are released and thrown into the

circulation, capillaries are dilated (CO_2 accumulates) and the diastolic blood pressure falls.

The increase in pressure will mean also more work for the left heart, because the margins of the valves will impinge upon each other more forcibly and as a consequence the marginal endothelium in such a region will be mechanically stimulated to a greater degree.

There are also coincident changes in the vessels of the valves. The pitressin injections which produce pressure response are very frequently followed by hemorrhage into the leaflets of the valves, causing considerable nutritive changes in the tissue of the valves.

After the pitressin injections we note the gradual changes in the valvular endothelium. The endothelial nuclei, at first, appear somewhat shorter and thicker. Their relation to the subendothelial layer changes with the thickening of the endothelial surface, on account of a thickening of the cytoplasm. Further, the cytoplasm of the endothelium definitely swells more and the nuclei no longer project above the surface. The nuclei become oval and round and later they appear elongated again, but perpendicular to valvular surface.

The smooth valvular surface also changes, becoming roughened and interrupted. Some of the endothelial cells appear to show vacuolization and their attachment to the subendothelial layer to be less firm. These vacuolated cells begin to project freely on the surface, being loosely attached to the subendothelial layer. The subendothelial cells are quite edematous.

Occasionally we actually find bacterial inclusions in heart valves in dogs that have had the induced pressor episodes.

The described changes in valvular endothelium cause the latter to become adhesive and bacteria circulating in the blood find an apportunity to adhere to the valvular surface and invade the valve. So if we, after pitressin, inject bacteria, we may see them adhering to the valvular surface and find them by cultural and bacteriological methods.

Cultural and bacterioscopical findings are accompanied by macroscopic lesions. At first a slightly elevated, hyperemic round lesion of a size from pinpoint to that of the head of a pin is found on the valvular surface. This type of lesion may be multiple and confluent. Later button-like excrescences and widely spread hemorrhagic zones appear. Finally small vegetations and ulcers develop.

If no bacteria were introduced after the pitressin injection, in a vast majority of cases the dogs appeared healthy, showing no signs of illness (except the reactions immediately after pitressin injections). But in some of the killed animals macroscopic and microscopic changes were observed.

At first a hemorrhagic lesion is found in the subendothelial area. The surrounding tissue appears swollen and edematous, with dilatation of vascular channels. An appearance of round cells and initial stage of fibroblastic proliferation are noted. Later, beneath the valvular surface there are observed a congregation of small and large mononuclears and fibroblasts. Some of the cells show vesicular nuclei. These cell clusters resemble an Aschoff nodule. We also observe typical palisades of fibroblasts, perpendicular to the valvular surface. Some of these fibroblasts proliferate above the valvular surface in a manner resembling the initial shape of verruca.

If the dogs are sacrificed in several weeks or months we find then congregations of scar tissue on the valvular surface in the form of a small verruca-

like formation. In some places the fibroblasts in palisade formation and perpendicular to the valvular surface bulged out of the valvular stroma, forming leaf-like bodies on the surface.

We also find bundles of scar tissue extending from the deeper tissue of the valve to the surface. Some of them are parallel to the valvular surface. There are also areas with no formed elements and only occasional round cells are observed. There are also found small verrucae whose lower portions are composed of fibrous tissue with few, if any, formed elements. At the base we might find bundles of scar tissue.

The fundamental and initial changes in the heart valves of animals with bacterial or non-bacterial endocarditis are the same in both cases. After mere pressor episodes, not accompanied by simultaneous bacteremia, the heart valve shows pathological changes resembling changes in rheumatic endocarditis. The bland endocarditis is a local reaction of a macroorganism to changes in its vascular status. The bacterial endocarditis represents a functional stage where bacterial adhesion and proliferation have been added to the primary change in endothelial status.

Discussion

(Dr. Alan R. Moritz, Cleveland.) I should like to ask Dr. Nedzel how he knew that the hemorrhage in the substance of the valve was from a vessel in the valve. We see hemorrhages in avascular portions of valves in man occasionally, and I would be interested to know how frequently the valves in dogs' hearts are vascularized.

(Dr. Benjamin J. Clawson, Minneapolis.) I should like to ask about the blood vessels deep in the valves, before you gave the inoculation with bacteria, and I should also like to ask about the interpretation of the pathogenesis of the disease, whether it develops outside the valve, or whether the infection begins deep in the valve. I was not certain from the sections whether the inflammation extended deep in the valve, as is commonly found.

(Dr. Isabella H. Perry, San Francisco.) Was the vitamin C intake in these animals lower in winter?

(Dr. Burton R. Rogers, Chicago.) I should like to ask Dr. Nedzel how he explains the heart beating 70 times a minute, and the flood of blood constantly washing over these valves, and the tenacity of these bacteria to stick on the free surface of the valve. Is there a possibility that they really come around when the aortic contracts and drives the blood into the coronary arteries and into the capillaries between the two transparent serous layers of the valve? Don't they really sneak in by the back door to the subserous side? I have often noticed over-exerted cattle driven rapidly, the whip and horses acting similarly to pitressin and increasing heart action, show endocardial congestion of valves and ventricles.

(Dr. Nedzel.) In many slides where hemorrhages were observed we could actually find many dilated blood vessels and we could see these hemorrhages coming out of blood vessels.

I did not look into the vitamin C deficiency. Our animals were fed well all year round. The animal hospital has an especially trained personnel and the animals have a well balanced diet. I do not think that the vitamin deficiency has been an important factor in our experiments.

In reply to Dr. Clawson's question about the blood vessels, I could not see them in the animals before injection of pitressin, because the observations were carried out after sacrificing the dogs. In control animals there were hardly any blood vessels seen. They were observed at the base of the valves, but after pitressin injections we could see dilated blood vessels also in other parts of the valves. I am sorry that time did not permit me to show these dilated blood vessels, but I may state that the vascular response even without the injection of bacteria was very noticeable. I am convinced that in my experiments the bacterial localization occurred from outside from the blood stream; bacteria, first settling on the valvular endothelium, gradually penetrated into deeper layers.

(Dr. Clawson.) About the inflammation of the valves?

(Dr. Nedzel.) In general we can say the heart valve *in toto* is edematous. I was primarily interested in the endothelial changes in the initial stages in connection with localization of the bacteria on the valvular surface. The said endothelial changes were of such nature that here was prepared a surface to which the bacteria could adhere and multiply. I think that also answers Dr. Rogers' question. In my experiments bacteria settled on the valves from the blood stream. They were constantly found on the valvular surface and did not come as emboli through the coronary blood vessels.

EXPERIMENTAL PULMONARY EDEMA AND CONGESTION. Sidney Farber, Boston, Mass.

Abstract. This report serves as an introduction to a study of neuropathic pulmonary edema in laboratory animals and in man. Section of both cervical vagosympathetic nerves initiates a series of changes which lead to death (Schiff, Traube, Schafer) usually within 2½ to 4 hours in the guinea pig (250 to 700 gm.) and 8 to 24 hours in the rabbit (800 to 3500 gm.). Severe pulmonary edema, congestion and sometimes hemorrhage are the most constant important findings. Consequences of the coincidental laryngeal paralysis (slow asphyxia, particularly in young animals; aspiration of secretions and food) may be superimposed on the pulmonary edema in variable degrees, giving a picture similar to that found in certain instances of poliomyeloencephalitis. Unilateral vagotomy causes neither pulmonary edema nor death. The survival time is prolonged (3 to 4 times) in the guinea pig if an interval of more than 10 days exists between section of the two nerves.

When the complications secondary to laryngeal paralysis are prevented (cannulation of the trachea or continuous artificial respiration) severe pulmonary edema still develops; the survival time is somewhat prolonged in the rabbit, but remains unaltered in the guinea pig. Laryngeal paralysis, therefore, is not an essential factor in the causation of pulmonary edema and death following double cervicalvagotomy. That the pulmonary branches of the vagosympathetic nerves are of primary importance is demonstrated by the occurrence of edema and death when only those branches are destroyed (guinea pig).

Preliminary studies of acute pulmonary edema in man indicate that disturbances to the vasomotor control of the pulmonary vessels, caused by either central or peripheral nerve disease, may account for alterations in the dynamics of the pulmonary circulation and in the integrity of the vessel walls. These

alterations are of importance in the pathogenesis of one form of pulmonary edema in man.

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I am extremely interested in Dr. Farber's presentation on pulmonary edema for the reason that a year ago it was my privilege to present before this Association the results of experiments on the production of edema. In these experiments agents were used that had the capacity of altering the permeability of the capillary walls. These agents included extracts of normal tissues, bile, sodium glycocholate, and narcotic substances, such as barbiturates. In each instance in which shock was produced and death was delayed, pulmonary edema was present. We came to the conclusion that the etiology of one type of pulmonary edema is integral with the etiology of shock. It has been our problem to determine the origin of shock when there were lesions of the central nervous system which had been observed. The shock syndrome may develop following a brain tumor, hemorrhage, or some other type of injury to the brain. Postmortem examination in such cases shows the same type of changes that we find characteristic of shock otherwise produced. This leads to the conclusion that one of the mechanisms whereby the vascular system may lose its tonus and congestion and edema of the lungs may develop, is of central nervous origin, or at least is bound up with the functioning of the central nervous system. I am therefore interested in the observation of Dr. Farber that permeability of the capillary walls was a mechanism in the production of pulmonary edema in his experiments. I am quite sure he is correct in that. I should like to ask whether barbiturate anesthesia was used in his animals. Our experiences have led us to abandon that type of anesthesia entirely in any type of experiment in which capillary tonus is a factor.

(Dr. H. Edward MacMahon, Boston.) Dr. Farber has told us that by cutting the vagus nerves bilaterally, pulmonary edema results. I should like to know if a unilateral pulmonary edema may be produced by cutting the vagus on one side only. Clinically and at the autopsy table one meets unilateral congestion, edema and hemorrhage at times following cerebral injury or associated with more severe operations. The explanation of these has been on a neurological basis and such terms as "neuropathic congestion," "neuropathic edema," and "neuropathic bleeding" are used. It would be of great interest if these conditions could be reproduced by mere unilateral section of the vagus nerve.

(Dr. Burton R. Rogers, Chicago.) I should like to ask Dr. Farber if he might also have cut the sympathetic nerves that go down the neck in the same sheath, on both sides along with the pneumogastric or vagus. They are considered antagonistic to the vagus.

(Dr. Farber.) I am well acquainted with Dr. Moon's interesting work on pulmonary edema. It should be emphasized that the experiments reported today deal with only one of the several causes of acute pulmonary edema. We have used barbiturate anesthesia in some experiments and we can verify what Dr. Moon has observed. Acute pulmonary edema was produced, however, under our experimental conditions when a variety of anesthetic agents were used, including local skin anesthesia. From experiments already carried out, but not reported today, it appears certain, in response to Dr. Moon's question,

that increased permeability of the pulmonary capillaries is of great importance in the production of this form of edema.

Dr. MacMahon asked about unilateral vagotomy. Unilateral vagotomy, either right or left, is followed by neither pulmonary edema nor death. The animals survive for many months without demonstrable changes in either the lungs or in general health. It is true, however, as we shall show on another occasion, that certain changes do occur following unilateral vagotomy which lead to pulmonary edema when other factors are introduced.

We have investigated the changes in the lung following thoracic sympathectomy and are continuing our work at the present time. I am not certain that it is correct to speak of a strong antagonism between the sympathetic and the parasympathetic nerves as far as vasomotor control of the pulmonary vessels is concerned.

MECHANISM OF INCREASED CAPILLARY PERMEABILITY IN INFLAMMATION. Valy Menkin, Boston, Mass.

Abstract. Previous studies of the writer have indicated the presence of a factor in various types of exudates which is capable of promptly increasing the permeability of the capillary wall (*J. Exper. Med.*, 1936, **64**, 485). The liberation of this substance by injured tissue, as evidenced by its consequent recovery in the exudate, has offered a reasonable explanation for the mechanism of increased plasma filtration into an inflamed area. The factor exhibited none of the manifest properties of histamine or of its presumably closely related H-substance, thereby rendering it difficult to accept the views of Lewis on the subject. The active substance was shown to be dialyzable and was recovered in a crystalline protein-free form.

An attempt was made to isolate the active factor relatively free of any gross impurities. Exudates were for the most part obtained from dogs after intrapleural injection with turpentine. However, in 1 case an exudate resulting from the intraperitoneal injection of aleuronat and starch in a rabbit was also analyzed for the presence of the active permeability factor. The cell-free exudate was treated with pyridine and the active principle was extracted after prolonged stirring of the precipitated mixture. This was eventually centrifugalized and the supernatant fluid was treated with several times its volume of acetone. The precipitate was centrifuged off and the supernatant fraction was evaporated to dryness *in vacuo*. Treatment of this residue with distilled water, followed by filtration and evaporation, yielded a practically homogeneous crystalline material. These crystals were characterized by a peculiar "branched and notched" pattern which sometimes assumed a "cross-like" appearance.

The crystalline material is extremely active (1:1000) in inducing a prompt increase in capillary permeability in rabbits when it is introduced intracutaneously, as evidenced by the rapid local accumulation of dye from the circulating blood stream. The crystals are very hygroscopic; they are soluble in water, acetone and aqueous alcohol, and relatively insoluble in ether and absolute alcohol. The material is heat stable. It yields a negative biuret test for proteins. The Molisch and Fehling tests for carbohydrates are negative. Unlike histamine (M. P. 130°C.) it shows no sharp melting point. At about 175°C. it chars and at 265°C. to 298°C. it still has not melted. The available evidence from proteolytic studies of total protein nitrogen and amino acid

nitrogen indicates that it is an intermediary product of protein catabolism. Its nitrogenous content averages 2.3 per cent. The pH of its aqueous solution may be slightly acid (6.5) or slightly alkaline to phenol red. It is precipitated out by saturated $(NH_4)_2SO_4$. It is readily dialyzable. It appears unlikely on the basis of accumulated observations that it is either a proteose, a peptone, or an amino acid. The data available at present seem to indicate that this active substance probably belongs to the group of polypeptides. Further work is being conducted in an endeavor to ascertain complete purity of the isolated substance and to determine its precise chemical structure.

This active substance recovered from exudates has in addition the interesting property of inducing a prompt aggregation and migration of polymorphonuclear leukocytes through the endothelial wall of capillaries. Within 20 to 40 minutes after its intracutaneous inoculation microscopic sections of the tissue reveal small vessels literally crowded with polymorphonuclear cells actively migrating outward. Furthermore, the substance is positively chemotactic. When placed in capillary glass tubes sealed at one end and introduced into an inflamed peritoneal cavity, the tubes soon become filled with leukocytes. This prompt effect on the migration of leukocytes following intracutaneous injection does not seem to depend primarily on its initial effect in increasing capillary permeability, for the introduction of turpentine, although causing an intense and prompt increase in endothelial permeation, fails to induce the rapid leukocytic migration observed in the case of the active substance. When normal blood is treated with the same extraction procedure a crystalline material is likewise recovered, but apparently in reduced concentration. It is only slightly active in inducing increased capillary permeability, and it barely calls forth any migration of leukocytes in equivalent intervals. The untreated exudate also induces a local cutaneous aggregation of leukocytes.

For the sake of convenience the name *leukotaxine* is tentatively proposed for this active crystalline nitrogenous substance which is evidently released by injured tissue and is readily recovered in inflammatory exudates. "Leukotaxine" *per se* is capable of rapidly initiating the usual sequences of the inflammatory reaction by first inducing a prompt increase in capillary permeability and secondly by causing an extremely rapid aggregation and migration of leukocytes through the endothelial wall.

Discussion

(Dr. Robert J. Parsons, New York City.) I should like to ask what the action of this material is when injected into the tissues with dyes or with diphtheria toxin. I am thinking of the Duran-Reynals factor.

(Dr. Menkin.) We have of course been very much interested to know whether the permeability factor in inflammation is related in any way to the Duran-Reynals factor, but we find that it is evidently an entirely different substance. The Duran-Reynals factor is non-dialyzable; it is heat-labile, whereas the permeability factor is dialyzable and heat stable. We have studied testicular extract and found that we could dissociate, by thermolability criterion, the two factors. We found, interestingly enough, that in normal testicular extract both factors are present — the Duran-Reynals factor and the permeability factor found in injury (*i.e.*, leukotaxine).

VASCULARITY OF THE BLOOD VESSEL WALL: PATHOLOGICAL CHANGES. Milton C. Winternitz and (by invitation) Robert M. Thomas and Philip M. LeCompte, New Haven, Conn.

Abstract. Intrinsic vessels arising from three separate sources are demonstrable in the aorta of the cow; they come from the adventitia, from the lip of the orifice of each branch, and also arise directly from the intima. These three groups anastomose freely within the wall of the vessel. Similar patterns are demonstrable in man and other animals. In normal young human vessels they are seen only occasionally, but with increasing age they become much more evident. They become conspicuous in physiological obliteration of vessels such as the ductus arteriosus and in the slow occlusion of the vessels in or adjacent to tuberculous cavities.

In the occlusion of the ductus arteriosus blood may be trapped in the lumen, or in the intrinsic vessels of the wall. This is the source of the concretions that are encountered as early as 4 months after birth. In the proliferation of the intima associated with the forms of disease that are included under the term arteriosclerosis, vascularity plays a conspicuous role. The number and size of blood vessels in the connective tissue matrix vary greatly. Blood may become trapped in these vessels, as occurs in the wall of the obliterated ductus arteriosus, to form concretions. Hemorrhages of varying size also occur and these lead in turn to the changes commonly classified as atheroma.

ENVIRONMENTAL FACTORS IN THE THROMBOTIC CONSTITUTION. William F. Petersen, Chicago, Ill.

Abstract. Apart from the factors in thrombosis that are generally accepted, such as changes in the vessel walls, in the blood constituents, and in the rate of flow, certain intangible and apparently unpredictable factors appear to take part in the constellation underlying the vascular condition. That seasonal forces must be considered has become evident from the statistics of the distribution of cases of thrombosis during the year; geographic considerations have revealed that thrombosis is rare in the tropics by comparison with the northern latitudes.

In a study of definitely dated thrombosis (retinal) in patients under continuous detailed chemical and clinical study over long periods of time, it has been made probable that an immediate environmental influence of the air mass plays a distinct role, particularly in providing vasomotor changes that are apparently summative in effect.

The precipitation of the thrombosis occurs definitely with the COD phase (*i.e.* the metabolic status that follows periods of unusually high blood pressure) when tissue anoxia has led to great periods of production of anoxybiotic acids, to capillary stimulation, and to dilatation of the vascular bed.

Such phase amplification occurs occasionally during the course of the repeated passage of abrupt polar fronts and when the reactive phase is augmented by an unusual rise in environmental temperature, the opportunity for thrombosis is definitely enhanced.

EXPERIMENTAL STUDIES ON THE INFLUENCE OF CARBON DIOXIDE ON THROMBOSIS AND OF CARBON DIOXIDE AND OXYGEN ON BLOOD CLOTTING. Joseph Tannenberg, Albany, N. Y.

Abstract. The studies concerning the influence of carbon dioxide on formation and progression of thrombosis were made on the exposed and narrowed inferior vena cava of rabbits. Beginning at various days following the operation (from the 1st to the 12th day) the rabbits were exposed to several periods of breathing mixtures of carbon dioxide. From one to five periods having the maximum duration of 8 hours were applied. The rabbits were killed at from the 2nd to the 18th day following the operation. Thrombi were found at the narrowed sites of the veins. The histological structure, however, showed that they were formed at one period referable exclusively to the time of operation. No additional proliferation corresponding to the breathing periods of carbon dioxide was present.

A new apparatus for the study of blood clotting under the influence of various gases was constructed which permitted keeping blood at constant temperature and under varying pressure of different gases without undue mechanical irritation during the clotting process. By means of this apparatus the influence of carbon dioxide and oxygen was studied on clotting of blood obtained from various sources. Gas pressure of from 10 to 120 mm. of mercury was applied. Carbon dioxide produced a marked delay of the clotting rate of from 30 to 50 per cent, but was not able to prevent the clotting entirely. Oxygen, even under pressure of 120 mm. of mercury, produced only a slight acceleration of clotting compared with atmospheric air. In additional experiments the influence of carbon dioxide on the agglutination of blood platelets was studied and found corresponding to the delay of blood clotting. The carbon dioxide is considered one, but not the only or most important, metabolic product that counteracts blood clotting and thrombosis, especially in the small veins of parenchymatous organs, such as the liver and kidneys, where the blood having passed two capillary systems is flowing particularly slowly.

ARTERIOLAR DISEASE IN HYPERTENSIVE AND NON-HYPERTENSIVE PATIENTS. Alan R. Moritz and (by invitation) M. R. Oldt, Cleveland, Ohio.

Abstract. The histological types, relative severity and distribution of chronic arteriolar lesions were investigated in all available tissues and organs from 200 autopsies. These 200 cases were comprised of two groups of 100 each, one group being of known non-hypertensive individuals and the other group of persons known to have had chronic hypertension. The tissues were examined objectively and the kind and severity of the changes in arterioles (100 μ or less in external diameter) were recorded.

The only portion of the body in which the presence of moderate or severe arteriolar sclerosis was almost invariably associated with evidence of chronic hypertension, whereas the absence of arteriolar sclerosis was almost invariably associated with evidence that chronic hypertension had not been present, was the kidney. Chronic arteriolar lesions were found in many situations in non-hypertensives and the severity of the lesions tended to increase with age. When, however, the arterioles of the kidney were affected in the same manner chronic hypertension was almost invariably associated. These findings indicated

either that chronic hypertension was caused by renal arteriolar disease, or that the renal arterioles had an unusually specific susceptibility to the effects of hypertension. If the examination were restricted to the non-hypertensive group it appeared that the renal arterioles were not especially predisposed to disease as compared with those of the spleen, pancreas and adrenals. If the occurrence of renal arteriolar sclerosis in hypertension represented effect of the hypertension, it would be reasonable to expect the renal arterioles to be susceptible to other injuries and to have a relatively high incidence of occurrence in non-hypertensive as well as hypertensive individuals, which was not the case.

The examination of tissues and organs where the presence of arteriolar disease was not necessarily accompanied by hypertension showed that in cases of hypertension both the incidence and severity of the arteriolar lesions were augmented. This was in accord with the clinical and experimental evidence that chronic hypertension may cause or augment arteriolar sclerosis.

This investigation did not disclose evidence that chronic hypertension was invariably associated with renal arteriolar disease, or that renal arteriolar disease invariably caused chronic hypertension. It did lend support to the view that in many cases of chronic hypertension the primary change was renal arteriolar sclerosis and was in accord with Goldblatt's experimental observations that reduction in blood flow through the kidney causes hypertension.

Three histological types of chronic arteriolar disease were identified and described. These were (1) intimal hyalinization; (2) medial hyperplasia; and (3) endothelial hyperplasia. These occurred in various combinations and were frequently altered by secondary degenerative changes. No type of chronic arteriolar lesion was found to be of and by itself pathognomonic of hypertension.

Discussion

(Dr. H. Gideon Wells, Chicago.) Dr. Moritz did not call attention to the striking similarity in his graphs of the arteries of the adrenal and kidney. That I think is pretty well recognized by pathologists in general. In routine examinations of autopsies, when I get the adrenal before the kidney, and have looked over the arteries of the adrenal, I can tell what I will find in the kidney. Dr. Moritz says that these changes in the kidney cannot be disregarded as indicating a relation of the kidney to hypertension, and you cannot very well leave out the adrenal either.

(Dr. E. T. Bell, Minneapolis.) Dr. Moritz has given an interesting explanation of this disease, and some splendid pictures of the structural changes in the vessels. I think that some of the differences of opinion among us are due to the fact that we draw the line differently on what an arteriole is. When one includes the small arteries with the arterioles he will find more arteriolar sclerosis than if he uses only the afferent glomerular arteriole. In all my studies I have used the afferent glomerular arterioles only, and that is perhaps the reason why I have found some cases of known clinical hypertension that did not have arteriolar sclerosis. My reports show that about 10 per cent of cases of clinical hypertension show no sclerosis of the afferent glomerular arterioles. If the arteriolar disease is first, and the hypertension secondary, how are we going to explain the 10 per cent with no arteriolar disease? On

the other hand, if you maintain that hypertension comes first and causes the arteriolar disease, you are still faced with the difficulty of explaining the cases where arteriolar disease is not present. Neither hypothesis escapes the dilemma. Dr. Moritz advances the explanation offered by Theodore Fahr that the primary disease is in the arterioles and that hypertension is secondary to arteriolar disease. Fahr asks: if the hypertension is primary, why does it affect only the kidney arterioles? But Fahr also assumes a hypersusceptibility of the renal arterioles to the agent that causes arteriolar sclerosis.

(Dr. Moritz.) I think that Dr. Wells' question might be answered in the same way if he had chosen the spleen instead of the adrenal. Severe arteriolar sclerosis occurs in the spleen and adrenals of hypertensive individuals, but it also occurs frequently in both organs in non-hypertensives. On the other hand, renal arteriolar sclerosis is rarely seen in non-hypertensives and in this investigation it was found almost invariably in cases of chronic hypertension.

In answer to Dr. Bell, I do not feel that disease in an arteriole of 100 μ in diameter is any less significant than disease in an afferent arteriole of 18 μ in diameter.

THE PATHOGENESIS OF ICTERUS NEONATORUM. Graham Ross (by invitation), Theodore R. Waugh and (by invitation) H. Tait Malloy, Montreal, Canada.

Abstract. A comparative study of the amount of blood destruction and bile pigment excretion during the 1st week of life of jaundiced and non-jaundiced infants was carried out. These investigations showed that:

1. The amount of excretion of bilirubin and urobilin in the stools during the 1st week was greater in the non-jaundiced cases.

2. The average level of the hemoglobin and total corpuscle volume of the blood on the 4th or 5th day was essentially the same in the two groups, though there were marked differences in individual cases.

3. The van den Bergh reaction on the blood plasma was always of the direct delayed type and quantitatively was proportional to the degree of jaundice.

4. Bilirubin was present in the urine in the cases showing jaundice and tended to appear when the bilirubinemia rose over four units.

5. The average fall in hemoglobin and total corpuscle volume from the 1st to the 5th day was essentially the same in both groups.

These findings offer a strong refutation to the hemolytic hypothesis of icterus neonatorum. Jaundice of the newborn is undoubtedly made possible by the blood destruction which occurs at birth. Nevertheless, whether a child does or does not develop jaundice is due to some factor other than the amount of destruction which occurs at this time.

Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) If one thinks of the state of the blood at a given moment as indicating a state of hemolytopoietic equilibrium, would it not be possible that Dr. Waugh's figures would be compatible with another situation, namely that although there is an excessive destruction of blood the levels are still equal, as were shown, because the destruction is

excessively well compensated for by increased bone marrow output? If this compensation were thought of as accomplished with little or no strain on the bone marrow, practically no change in the peripheral blood picture would have to be expected.

(Dr. Waugh.) In answer to Dr. Krumbhaar, there is no evidence in the blood in jaundiced or non-jaundiced babies, so far as I know, of any excessive hematopoietic activity in 1 case as compared with the other. As a rule, the nucleated red cells disappear quite rapidly, along about the 3rd or 4th days, and so far as I know, it has never been pointed out, although it may be possible, that there does exist any hematological difference.

AN EXPERIMENTAL STUDY OF THE EFFECT OF BENZEDRINE SULPHATE ON RATS. William E. Ehrich (by invitation) and E. B. Krumbhaar, Philadelphia, Pa.

Abstract. One hundred and seventy-one albino rats, weighing from 50 to 385 gm. were used. One hundred and forty-five were injected subcutaneously with doses of 1 to 500 mg. per kilo from 1½ to 6 weeks. The lethal dose decreased with the weight (age) of the rats. In animals of 50 to 95 gm. it amounted to 200 mg.; in rats of 100 to 195 gm., to 50 to 60 mg.; in rats of 210 to 385 gm., to 30 to 35 gm. per kilo. After repeated injections a slightly increased tolerance was noted. If 5 mg. and more were given the rats showed excitement, diarrhea, mydriasis, transient inhibition of weight increase, erythrocytosis, leukocytosis, and so on. With 25 mg. and more they showed an increase in glycogen and a decrease in fat in liver and fat tissue. In rats that died from the drug frequent changes were constrictions of the small intestine, congestion of the liver, either marked constriction or congestion of the spleen, and subpleural hemorrhages in the lungs. Rats that died of from 30 to 100 mg. of benzedrine showed necroses in liver, spleen and kidneys, whereas those that died from higher doses did not. Lesions in the myocardium, arterial or intestinal walls, such as are observed in chronic adrenalinism, were not found.

INHIBITION OF THE SHWARTZMAN PHENOMENON IN LYMPH NODES. Lewis Henry Koplik (by invitation), New York City.

Abstract. Gross (*Zentralbl. f. Bakt.*, 1. Abt., Orig., 1931, **122**, 96) and Ogata (*J. Exper. Med.*, 1936, **63**, 59) observed that the intravenous injection of a potent bacterial filtrate given within a certain period of time prior to or following the skin preparatory injection produced an inhibition of the Shwartzman phenomenon. The association of hemorrhage and thrombosis in regional lymph nodes with the Shwartzman phenomenon has already been described (Koplik, L. H., *J. Exper. Med.*, 1937, **65**, 287). It was pointed out that this reaction, while limited to the regional nodes, may be independent of hemorrhage at the injected skin site, the lymph nodes being more susceptible to the production of the Shwartzman phenomenon than the areas of skin which they drain. The purpose of the present work was to study the reaction in regional and distant lymph nodes of rabbits in which the inhibition of the Shwartzman phenomenon had been attempted according to the method outlined by Ogata.

Stock rabbits were given an intravenous injection (1 cc. per kilo) of dilu-

tions of meningococcus (Shwartzman, G., *J. Infect. Dis.*, 1929, **45**, 232), *B. coli*, or *B. typhosus* culture filtrate (Shwartzman, G., *Proc. Soc. Exper. Biol. & Med.*, 1929, **26**, 843) immediately preceding the skin preparatory injection for the Shwartzman phenomenon. The dilution of meningococcus culture filtrate was equivalent to 20 to 25 reacting units per cc., that of the *B. coli* and *B. typhosus* culture filtrates varied between 1:10 and 1:75 depending on the strain and filtrate used. Meningococcus culture filtrate was used for the production of the Shwartzman phenomenon, 0.25 cc. of a 1:25 dilution for the preparatory injection and 20 to 25 reacting units for the provocative intravenous injection given 20 to 24 hours later. Four to 5 hours after this second intravenous injection the skin reactions were read and the animals were killed by ether. They were autopsied, and regional and distant lymph nodes were removed for study. Control rabbits were similarly treated for the production of the Shwartzman phenomenon but did not receive the preliminary inhibitory injection of filtrate.

Of 16 control rabbits 15 showed gross and microscopic hemorrhage and all presented extensive thrombotic changes in the veins and capillaries of the regional lymph nodes. The injected skin sites were negative in 5 instances; in 11, the Shwartzman phenomenon was present. Distant lymph nodes were normal.

In contrast, rabbits given an intravenous injection of 20 or 25 reacting units of meningococcus culture filtrate just before the skin preparatory dose showed neither hemorrhage nor thrombosis in the regional nodes in 7 of 9 animals so treated. The skin reactions were inhibited in 5 of these rabbits. Distant nodes were negative in all cases.

Inhibition of the Shwartzman phenomenon in regional lymph nodes was also observed in rabbits given an intravenous injection of heterologous filtrate (*B. coli* or *B. typhosus*) just before the skin preparatory injection. Certain concentrations of filtrate in the inhibitory injection, depending on the strain and filtrate used, were necessary for inhibition of the phenomenon. Thus, with a dilution of 1:30 of a filtrate of *B. typhosus* (strain TL) culture lymph nodes were negative in all of 4 rabbits, while a dilution of 1:75 evoked no inhibition in 6 others. In these experiments the skin reactions were also inhibited though not so regularly or completely as those in the lymph nodes.

The experiments demonstrate that it is possible to prevent the appearance of thromboses and hemorrhage in regional lymph nodes by an inhibitory intravenous injection of a bacterial filtrate just prior to the preparatory intradermal injection. Just as there is a lower threshold for the production of the Shwartzman phenomenon in lymph nodes than in the skin, so the results of the present experiments are suggestive of an increased susceptibility of these nodes to an inhibition of this reaction. Certain quantitative relations are necessary for this inhibition. It is not specific but is associated with the potency of the inhibiting filtrate as regards its capacity to elicit the Shwartzman phenomenon.

Discussion

(Dr. Howard T. Karsner, Cleveland.) It has been instructive to hear this interesting paper by Dr. Koplik and I wish to compliment him upon the work. Although it is difficult to interpret lantern slides, I wish to ask if the slide which he showed as illustrative of a normal lymph node from the region

of a preparatory injection does not actually show infiltration of leukocytes. The interpretation of inhibition of reaction in the lymph nodes involves determination of the reaction quantitatively. The reaction consists of various components, such as hyperemia, edema, thrombosis, hemorrhage, necrosis and infiltration by leukocytes. In order to express the reaction quantitatively it seems necessary to give each of these its own weighting, unless each were considered to be of equal significance. It would be of interest to have Dr. Koplik explain how he arrives at the quantitative evaluation of the reaction in skin, subcutaneous tissues and lymph nodes.

(Dr. Max B. Lurie, Philadelphia.) It is difficult for me to understand how the intravenous administration of the active filtrate 24 hours previous to the administration of the preparatory factor can inhibit the Shwartzman reaction when the reacting factor is later introduced intravenously. However, is it possible that Menkin's observation will explain this inhibition? He found that if trypan blue is introduced next to an area of inflammation of a certain duration the dye will not penetrate the site of inflammation. I wonder whether the introduction of the active filtrate produces the same change in the permeability of the blood vessels, so that later when the preparatory factor is introduced into the skin it will not penetrate into the blood vessel wall, thus interrupting the chain of events leading to the Shwartzman phenomenon.

(Dr. M. J. Shear, Boston.) The filtrates used contain a wide variety of substances. The histological changes might perhaps be clarified by producing the Shwartzman phenomenon with a purified agent that was separated from accompanying contaminants. It has been found possible to separate a fraction from *B. coli* filtrates which produces hemorrhage in the skin of rabbits in doses of 0.0005 of a milligram. Pathologists may find it of advantage to employ the purified agent in studies such as this.

(Dr. Koplik.) In answer to Dr. Karsner, it is perfectly true that there is a leukocytic infiltration when an intradermal injection only is given. It is also true that this infiltration is proportional to the concentration of the filtrate used intradermally. However, the amount of inflammation following preparation does not seem to bear any direct relation to the degree of hemorrhage or thrombosis that may be obtained after the subsequent intravenous injection.

With reference to a quantitative relation I think we might say from these experiments and from my previous work on the phenomenon in lymph nodes that perhaps the earliest lesion to appear is the vascular thrombosis. When the phenomenon is more marked we also get hemorrhage. Hemorrhage may be present with relatively little thrombosis, but in the present inhibition experiments the lesions in the lymph nodes which were visible only microscopically did not show hemorrhage but merely thrombosis.

As to Dr. Lurie's question regarding the permeability of the blood vessels, we have not thus far investigated the mechanism of inhibition.

In answer to Dr. Shear, the experiments on inhibition have not been done with fractions of the filtrates but only with the filtrates themselves. It seems essential that filtrates containing the Shwartzman factors themselves be employed in order to produce inhibition. Ogata failed to obtain inhibition with plain broth, peptone and streptococcus filtrates devoid of the Shwartzman factors.

THE REYNALS FACTOR IN FETAL AND ADULT GUINEA PIG TISSUES. N. Paul Hudson and (by invitation) O. N. Fellowes, Columbus, Ohio.

Abstract. In a study of the marked susceptibility of the guinea pig fetus to vaccinia virus (Stritar and Hudson), we have attempted to determine whether there is an extractable factor peculiar to fetal tissues capable of enhancing the action of the virus in rabbit skin. Such a principle, found by Duran-Reynals and others to be in highest concentration in the rabbit testis, has been called "the Reynals factor."

Saline extracts of fetal and adult guinea pig tissues were mixed with decreasing amounts of vaccinia virus carried in rabbit testicular tissue. The mixtures were inoculated intracutaneously and the appearance and size of lesions recorded at the end of the 6th day.

The ratios between the sizes of the virus control and test lesions were: fetal kidney 1:2.3, adult kidney 1:1.9; fetal liver 1:1.8, adult liver 1:1.7; fetal brain 1:1.9, adult brain 1:1.7; fetal skin 1:2.3, adult skin 1:1.7; fetal lung 1:1.7, adult lung 1:1.7; normal rabbit testicle 1:2.8.

Apparently there is no extractable property capable of enhancing the action of vaccinia virus, which is peculiar to the tissues of guinea pig fetuses. The transmissible factor is present in fetal and adult tissues, but not to the degree had by the rabbit testis. While the Reynals factor may be significant in the greater susceptibility of the fetus to vaccinia virus and be outweighed by some other factor, it does not appear to be the sole demonstrable basis for susceptibility.

Discussion

(Dr. A. E. Casey, St. Louis.) I should like to ask Dr. Hudson whether the term "Reynals-McClean factor" would be equally acceptable? McClean in England reported at about the same time as Duran-Reynals.

(Dr. Hudson.) I should like to give expression to the fact that McClean described some very fine studies. I hope we will be able to learn some more about this factor in its definition, but since we measure it here by its action, and its chemical nature has not been described, we might still use the name given. It might be well to use the names Reynals and McClean together.

THE DEVELOPMENT OF THE LOCAL CELLULAR REACTION TO TUBERCULIN IN SENSITIZED CALVES. William H. Feldman, Rochester, Minn., and (by invitation) C. P. Fitch, St. Paul, Minn.

Abstract. Using a group of sensitized calves a study was made of the cellular reaction that follows the intracutaneous injection of tuberculin. After the tuberculin was injected biopsies were done at 6 to 8 hour intervals up to and including the 72nd hour and on the 5th, 7th, 10th and 14th days. The reactive process showed a constant selection for the perivascular and perineural tissues and during the early phases of the reaction polymorphonuclear leukocytes were numerous, while eosinophilic granulocytes and histiocytes were in the minority. There was a gradual but constant increase in the histiocytes up to the 72nd hour. This was particularly noticeable at and beyond the 30th hour. The polymorphonuclear leukocytes gradually diminished and between the 60th and 72nd hour the histiocytes predominated. Acidophilic polynuclear and

mononuclear granulocytes were usually numerous. The character of the reaction persisted practically unchanged up to the 14th day although most of the cells other than histiocytes had disappeared. The histiocytes in the tissue removed on the 14th day were richer in chromatin and many were becoming oval or fusiform in contour, suggesting fibroblasts. Other features of the process consisted of edema and endovascular changes such as venous thrombosis and endarteritis. Lymphocytes and plasma cells assumed a minor role in the reaction.

Discussion

(Dr. Max B. Lurie, Philadelphia.) I should like to ask Dr. Feldman whether his study showed any evidence for the differentiation between the anaphylactic and so-called tuberculin type of reaction. Mallory and Dienes claimed that in the tuberculin reaction, if a small enough dose of tuberculin is given, the reaction is almost completely mononuclear in type from the very beginning, whereas in the anaphylactic reaction polymorphonuclears are the most predominant. I should like to ask Dr. Feldman if he gave these calves a sufficiently low dose of tuberculin to answer this question.

(Dr. Virgil H. Moon, Philadelphia.) I should like to ask whether Dr. Feldman has an explanation of the mechanism by which edema develops in these reactions. Edema is, of course, a feature of inflammatory reactions. Does Dr. Feldman feel it has the same mechanism here as in inflammatory reaction in general?

(Dr. E. R. Long, Philadelphia.) Apropos of Dr. Lurie's remarks, Dr. Vorwald, Dr. Holley and I in experiments in Chicago obtained results that conform entirely to those Dr. Feldman just reported. We have not seen the striking difference between the anaphylactic type of response and the tuberculin type of response that Mallory and Dienes reported. On the contrary, we have seen exactly what Dr. Feldman reported, a polymorphonuclear reaction beginning in a few hours and lasting up to 2 days, followed by replacement of the polymorphonuclear cells by mononuclears. I have thought that some difference in the inoculation dose might have produced the different results. I think what Dr. Feldman reported holds for small doses.

(Dr. Feldman.) We have not attempted to verify or investigate the anaphylactic reaction reported on by others. Our dosage of tuberculin was that usually used by the Bureau of Animal Industry of the U. S. Department of Agriculture. We used 0.4 cc. for each injection; the solution representing 25 per cent of old tuberculin.

As regards Dr. Moon's question, I cannot offer any explanation. The edema does not seem in any way different in these inflammatory reactions than it does elsewhere.

EXPERIMENTAL STUDIES ON POSSIBLE MECHANISMS OF CERTAIN PREDISPOSING FACTORS TO LOBAR PNEUMONIA. W. J. Nungester and (by invitation) R. G. Klepser, Ann Arbor, Mich.

Abstract. It has been shown that sterilized gastric mucin or sputum introduced deep into the lower respiratory tract of the rat along with small quantities of pneumococci aided in producing lobar pneumonia. The question was raised as to whether or not certain predisposing factors to pneumonia might

"lower the resistance" by causing the aspiration of mucinous secretions from the upper respiratory tract to the lungs.

Exposure to cold or marked alcoholic intoxication are recognized as predisposing factors to pneumonia. These factors have been investigated from this point of view. Mucin and India ink were placed in the noses of rats under a light ether anesthesia. Some of the rats were intoxicated with alcohol, others were sprayed with cold water, and others held as controls. About 20 animals were included in each group.

Only microscopic amounts of ink were noted in the lungs of the control animals sacrificed 1 hour after inoculation; 55 per cent of the intoxicated animals and 53 per cent of the rats exposed to cold had gross amounts of ink in the lungs. 10^{-4} cc. of a type I pneumococcus culture were substituted for ink in an analogous experiment. From 24 to 47 animals were included in each group. Thirteen per cent of the control animals developed lobar pneumonia. In the intoxicated group 38 per cent were found to have pneumonia, while 42 per cent of the rats exposed to cold water developed lobar pneumonia. Cold and alcohol appear to interfere with the function of the epiglottis. Aspiration of large amounts of culture without mucin did not result in pneumonia in any of 42 rats.

Discussion

(Dr. Francis G. Blake, New Haven.) I do not think anyone would take exception to the view that the introduction of mucin or other substances of that type may predispose in certain animals to the development of pneumonia. What bearing this may have on the development of pneumonia in man, is, of course, difficult to say. In this connection it should be recalled that 100 per cent of Filipino monkeys develop typical lobar pneumonia following intratracheal injection of 0.000001 cc. of *Pneumococcus* Type I culture without mucin or other added factors.

(Dr. Nungester.) We would not want to draw any conclusions on the pathogenesis of pneumonia in man. Mucin in the tract is not absolutely necessary because we can produce pneumonia by large doses of pneumococci injected without mucin, but in a rather low incidence of cases. We believe other factors may favor the development of pneumonia in the lungs. Two such factors we have investigated are serum and plasma. Both of these when introduced into the lung along with pneumococci give a higher incidence of pneumonia than when pneumococci are introduced with 0.85 per cent saline.

THE RESULTS OF INTRATRACHEAL INJECTION OF BORDET-GENGOU BACILLI IN MONKEYS AND RABBITS. D. H. Sprunt and (by invitation) D. S. Martin and Sara McDearman, Durham, N. C.

Abstract. Recently several workers have reported the production of a lymphocytosis and a paroxysmal cough or whoop in apes by the injection of large numbers of the Bordet-Gengou bacilli. As we had previously reported interstitial mononuclear pneumonia resulting from the injection of the Bordet-Gengou bacillus in rabbits, we thought a further study of the morbid anatomical and the blood changes in both the monkey and the rabbit would be of interest.

Six *Cebus* monkeys and a number of rabbits were inoculated intratracheally with virulent Bordet-Gengou bacilli. Five of the monkeys and all of the

rabbits had a marked lymphocytosis and an interstitial mononuclear pneumonia. The lymphocytosis was shown to be significant as it was more than double the maximum reached in 91 counts on normal monkeys.

The Bordet-Gengou bacillus could be recovered by daily nasal cultures from any of the animals but was cultured at autopsy from only 1 monkey.

These observations indicate that both the interstitial mononuclear pneumonia and the lymphocytosis are the result of a toxin liberated by the Bordet-Gengou bacillus in the tissues and agree with the idea that the cough is the result of the multiplication of the bacilli on the cilia. A reasonable explanation of the pathogenesis of pertussis in apes is that a number of the bacilli disintegrate and produce an interstitial mononuclear pneumonia which serves as suitable substratum for the organisms to multiply and cause the whoop.

The enormous dose of Bordet-Gengou bacilli required to produce the described change suggests that another agent may be required to initiate the Bordet-Gengou infection in man; as viruses produce such a reaction it may be, as has been suggested by other workers, that infection with the Bordet-Gengou bacilli is dependent on a preceding virus reaction in the lungs.

Discussion

(Dr. A. E. Casey, St. Louis.) I wonder whether Dr. Sprunt differentiated between monocytes and lymphocytes. In most diseases it is the monocyte that rises in the first week or 10 days, and the lymphocyte only after some weeks.

(Dr. Howard A. McCordock, St. Louis.) These experiments and those that preceded them from the same laboratory are of great interest to those who have concerned themselves with the peculiar pneumonias that follow certain acute infectious diseases. I am glad that Dr. Sprunt said that the pneumonia following pertussis is slightly different from the experimental lung lesions produced by massive doses of bacteria. Any adequate explanation of this problem must account, not only for the interstitial reaction which is the subject of this paper, but must also take into consideration the hemorrhagic pneumonia that often precedes it, as well as the subsequent complications such as lung abscess and empyema, all of which are associated with the infectious diseases in which interstitial pneumonia is commonly found. The acute, fulminating hemorrhagic and edematous pneumonia that occurs in those individuals who die within a few days after the onset of symptoms of epidemic influenza, is as much a part of the problem as the later interstitial pneumonia. It has been shown experimentally that one can produce all these lesions either with a virus alone or with a virus in combination with some bacteria. That is more than can be accomplished with bacterial organisms acting by themselves.

In our first publication on virus pneumonia we pointed out that many bacterial, as well as substances other than viruses, may produce an interstitial reaction in the lung. In spite of this fact we regarded interstitial bronchopneumonia as a fairly reliable histological index of virus action and I still believe it is. An acute inflammatory exudate is a useful index of the presence of pyogenic bacteria in tissue, even though it may also be called forth by sterile necrotic tissue or chemical irritants. Tubercles are produced by many foreign bodies other than the tubercle bacillus, and yet tubercles are helpful in the diagnosis of tuberculosis. Similarly it would be most unusual for an

interstitial reaction in the lung to be reserved exclusively as a defense against viruses. To infer that interstitial bronchopneumonia is useless as an index of virus action because wholesale doses of bacteria of low virulence also produce an interstitial reaction is to ignore all the other aspects of the problem.

Several investigatiors have shown experimentally that *B. influenzae* may produce an interstitial reaction and that large quantities of fluid cultures containing toxin may call forth a hemorrhagic and edematous pneumonia. However, interstitial bronchopneumonia is seen only in epidemic influenza, with which a virus is associated, and never in other pulmonary conditions in which *B. influenzae* may be recovered from the lung tissue.

No one from our laboratory has ever claimed that whooping cough was caused by a filterable virus and that the Bordet bacillus may be ignored in considering the etiology of this disease. We are interested in the high incidence of intranuclear inclusions in the lungs in pertussis and are seeking the explanation of their presence. To date, we have demonstrated them both in our St. Louis material and also in sections from other cities, in about 30 per cent of all cases of pertussis so far examined. They cannot be produced by bacteria. This phase of the pertussis problem can never be solved by repeating experiments with the hemophilic bacilli.

The coughing in pertussis can hardly be attributed to the sticking of bacilli between the cilia of the bronchial epithelium. This is also seen in a variety of other conditions. Influenza bacilli can be demonstrated in a variety of lung lesions in the same situation and yet these individuals have no paroxysms of coughing. It has always seemed to me that the presence of lesions in ganglia at the root of the lung explains the paroxysmal character of the cough better than the mechanical irritation of the cilia of the bronchial mucosa.

(Dr. Sprunt.) In answer to Dr. Casey, we included no monocytes in the lymphocyte counts.

I cannot, at this time, answer any of the points raised by Dr. McCordock except to point out that although a virus may cause an interstitial mononuclear pneumonia, there are also a number of other agents that may cause this same type of reaction. We have previously shown that bacterial toxins like viruses when used in large amounts cause a hemorrhagic pneumonia and when used in smaller amounts cause an interstitial mononuclear pneumonia. In closing I would emphasize that the presence of an interstitial mononuclear pneumonia does not justify definite conclusions as to the nature of its incitant.

THE CHEMOTROPIC ATTRACTION OF LEUKOCYTES BY FRACTIONS OF *Streptococcus haemolyticus*. H. M. Dixon (by invitation), Morton McCutcheon, and (by invitation) E. J. Czarnetzky, Philadelphia, Pa.

Abstract. From experiments reported before this Society last year, it was concluded that the chemotropic attraction of polymorphonuclear leukocytes to bacteria *in vitro* is brought about by substances given off by the bacteria. The present experiments represent an initial step in identifying such substances, by testing the chemotropic effect *in vitro* of certain fractions of *Streptococcus haemolyticus*.* These fractions were: (1) a labile antigen con-

* For the method of preparation of these fractions see Mudd, S., Czarnetzky, E. J., Pettit, H., and Lackman, D. Labile bacterial antigens and methods for their preparation and preservation. *Proc. Amer. Philos. Soc.*, in press.

sisting of a protein-carbohydrate complex, which, on injection into rabbits, produces specific agglutinating and phagocytosis-promoting antibodies; (2) a protein-free, non-antigenic, crystalline, stable hemolysin; and (3) a carbohydrate (fairly pure and freed of protein). Each of these three substances was adsorbed on kaolin (which by itself does not attract leukocytes) and was then tested as a source of attraction of rabbit polymorphonuclear leukocytes *in vitro*. The leukocytes were obtained by injecting physiological saline solution into the peritoneal cavity; the exudate was mixed with plasma and a drop of the mixture was allowed to spread between slide and coverslip, with the coated kaolin particles in the center. Under the microscope it was observed that leukocytes were strongly attracted to the particles coated with the labile antigen, but not to those coated with the other two fractions. It is concluded that of the three fractions of hemolytic streptococcus tested, the one that calls forth phagocytosis-promoting antibodies is also the substance that attracts leukocytes to the bacteria, thus making possible their phagocytosis.

BIOLOGY OF THE INFECTIOUS AGENT OF TRACHOMA. Louis A. Julianelle, St. Louis, Mo.

Abstract. In pursuing the tentative concept that the infectious agent of trachoma may be a virus, studies have been conducted on its nature as determined by its behavior under different conditions. Since experimental trachoma may be induced in monkeys with material free from cultivable bacteria, as may be demonstrated by rabbit testicular passage of trachomatous tissues, a concerted effort has been made to cultivate the infectious agent in tissue culture. For this purpose a number of technics have been employed, including tissue cultures of 6 animal species (chicken, mouse, guinea pig, rabbit, monkey and man), without, however, demonstrating propagation. Filtration of trachomatous tissue through Berkefeld V, collodion membrane (a.p.d. *ca.* 0.6μ), Seitz and plaster of Paris (Kramer) filters indicates that, regardless of the method used, the infectious agent is so rarely filterable as to make this method of study impracticable. The influence of various agents, physical and chemical, reflects the extremely delicate nature of the infectious agent.

Studies on immunity to trachoma reveal that no active immunity is demonstrable following recovery from experimental infection. So, also, it was not possible to detect protective antibodies in the blood of patients or animals experimentally infected.

LOCAL PRODUCTION OF ANTIBODIES IN VACCINIA. O. N. Fellowes (by invitation) and N. Paul Hudson, Columbus, Ohio.

Abstract. Considerable attention has been given the problem of local production of bacterial antibodies (Cannon and associates), but little has been done in connection with viruses (Holden and Strong, McMasters and Kidd). We have investigated the production of so-called neutralizing antibodies in the skin of rabbits inoculated dermally with vaccinia virus.

Extracts of the skin lesion areas were made by the process of freezing and thawing. Twenty per cent extracts were mixed with diminishing quantities of virus carried free of bacteria in the rabbit testicle. Antibodies were measured by the prevention of cutaneous lesions in the rabbit in terms of the

amount of virus neutralized. The tests were conducted on skin areas bearing lesions from 1 to 30 days old. Parallel control examinations were made with the serum of a normal rabbit, a known positive serum from a convalescent rabbit, the serum from the animal furnishing the skin lesions, and extracts of normal rabbit skin and of the uninoculated skin of the rabbit having the skin lesions.

Neutralizing antibodies first appeared in the serum on the 3rd day after inoculation, mounted to the level of the convalescent serum control on the 12th day, and dropped in titer from the 16th day. Extractable antibodies were demonstrable in the skin lesion areas on the 4th day, increased in titer to the 20th day and persisted throughout the experimental period of 30 days. Antibodies appeared in uninoculated skin on the 9th day, but were not tested for after the 12th day. Normal serum and extracts of normal skin did not neutralize the virus.

Discussion

(Dr. George Hartley, Jr., Chicago.) In connection with this interesting work I should like to present some evidence on the local antibody formation of antivaccinial antibodies in rabbit skin that agrees in general with the above. To obtain satisfactory proof of the local production of antibacterial antibodies it has been found necessary in this laboratory to first irritate the local area, either specifically or non-specifically. This irritation calls forth, along with other cells of inflammation, an infiltration of macrophages. Since these cells are important factors in the production of antibacterial antibodies, it was assumed that they are probably also necessary for the production of antiviral antibodies. Accordingly we first irritated the skin with a single intradermal inoculation of $Al(OH)_3$ gel prepared by Willstaater's, Type C formula for enzyme purification. This irritant has a three-fold advantage. In the first place, it is an excellent macrophage mobilizer; great numbers infiltrate the area and phagocytose the gel by the 4th or 5th day following inoculation. Secondly, the vaccinia virus is readily adsorbed to and is not inactivated by the $Al(OH)_3$ gel, thus preventing to a great extent virus generalization. And thirdly, the lesion becomes quite avascular after 2 to 3 weeks, also decreasing the chance of virus generalization.

Some 18 to 21 days following the $Al(OH)_3$ inoculation a small quantity of virus, ranging from 0.5 to 1.5 skin infecting doses in 0.04 cc. amounts is adsorbed to Al $(OH)_3$ and inoculated directly into the nodule. Three and one-half to 4 days later the animal is sacrificed and the virus inoculated nodule, together with a normal control nodule of skin, blood, spleen, liver and bone marrow, is removed, extracted and filtered through Seitz asbestos pads to remove all traces of virus that might be present in the tissues.

The results of these experiments show that 10 per cent extracts in saline of the local virus inoculated area contain in 3½ to 4 days approximately three times as many neutralizing antibodies as the whole blood serum and control skin area, twice as many as the spleen, and ten times as many as the liver and bone marrow. In about one-third of the animals the whole blood serum contained no demonstrable neutralizing properties whatsoever. In all cases but 1 the local virus inoculated area contained as high or higher concentration of virucidal antibodies as did the whole blood serum. The series includes approximately 25 animals. We believe, therefore, that when vaccinia

virus is adsorbed to $Al(OH)_3$ gel and inoculated into a locally prepared macrophage area of skin, which also contains $Al(OH)_3$, most of the virus is held locally and after about 4 days the macrophages have produced demonstrable neutralizing antibodies which have not as yet, in most cases, been poured out into the blood stream.

(Dr. Max B. Lurie, Philadelphia.) A somewhat similar phenomenon is observed in tuberculosis and, to my mind, it appears that the same principle is involved. If tubercle bacilli are injected into a local area subcutaneously, it is well known that they multiply there and that some are disseminated throughout the body. When immunity sets in, the destruction of the bacilli takes place first and to a marked degree at the point of injection. Some destruction, and to a lesser degree, takes place at the same time in the draining lymph nodes immediately bordering on the lesion. Yet at this time multiplication goes on uninterrupted in the internal organs. While this is no evidence of local antibody production, it is evidence of greater immunity being produced at the point of injection of the organism and of less immunity being present in other parts of the body, and that this immunity decreases in proportion to the distance removed from the point of the first introduction of the bacillus.

(Dr. Paul R. Cannon, Chicago.) We have been hampered in the demonstration of the production of antibacterial antibodies by the complicating factor of non-specific flocculation. Last year when Dr. McMaster reported on the local formation of antibodies to vaccinia in lymph nodes, we thought that a neutralizing method might get around the difficulty of non-specific flocculation.

Dr. Walsh and I have been studying the problem of local formation of antibacterial antibodies in the respiratory tract. Our general method is as follows: We immunize the same animal locally in the respiratory tract with *B. typhosus* and intraperitoneally with *B. paratyphosus B.* We sacrifice the animals at varying intervals and titrate the tissue extracts and blood serum against both antigens. In this way we can control the non-specific flocculating factor as well as obtain a ratio of titers of tissue to blood serum for both antibodies. We have found in some instances that the titer of blood serum was identical for both *B. typhosus* and *B. paratyphosus B.* The ratio of antibody titer of respiratory tissue to blood serum, however, might be 1:1 with respect to the bacteria used for local immunization, whereas it was 1:8 for the microorganism used in the general immunization. Such an unequal concentration of antibodies in the same tissue extract, with the concentration in the blood serum being equal, is strong evidence for the local formation of antibodies as a result of local antigenic stimulation.

THE ACTION OF IMMUNE SERUM UPON INFLUENZA VIRUS *In Vitro.* T. P. Magill (by invitation) and Thomas Francis, Jr., New York City.

Abstract. Studies have been conducted on the effect of immune serum upon a strain of human influenza virus (PR8) grown in chick embryo tissue culture medium. The results show (1) that when cells are exposed to the action of immune serum of low titer and subsequently washed, they support the growth of virus as well as cells treated with normal serum; (2) that, in agreement with the results of other workers, when virus is added to cell sus-

pensions before the addition of immune serum of low titer, virus survives in the cells; and (3) when mixtures of immune serum of low titer and virus are added to cells there is little evidence of survival or multiplication of the virus. Furthermore, when immune serum of high titer is used the virus is inactivated irrespective of whether the cells are first exposed to virus or immune serum. Finally, virus mixed with strong immune serum is inactivated in the absence of cells as shown by the fact that centrifugation at high speeds of such serum-virus mixtures yields no active virus, whereas normal serum-virus mixtures yield fully active virus.

A CANINE ENCEPHALITIS WITH SOME SPECIFIC CHARACTERS. Robert G. Green, Minneapolis, Minn.

Abstract. A beagle hound about 5 months old suddenly exhibited great excitement and fear, ran about wildly, and finally fell over unconscious. A few days later a similar attack was followed by marked choreiform movements. The animal was not gravely ill when etherized and killed. Microscopic examination of tissues showed principally large acidophilic inclusions in the nuclei of the smaller nerve cells of the brain. A few affected cells occurred throughout the brain, but definite foci of inclusions were found in the cerebellum. The typical inclusion is a large uniform body, occupying about half the nuclear space and separated from the nuclear wall by a clear zone. A fine margination of chromatin is typical, but occasionally the chromatin is attached to the nuclear wall in large masses. Attempts to transmit the infection by brain emulsion to both dogs and foxes have given negative results. The large number of nerve cells involved indicates that the pathological change observed was the basis of the symptoms produced, and the typical appearance of the intranuclear inclusions suggests a virus origin of the disease.

Discussion

(Dr. Howard A. McCordock, St. Louis.) I should like to inquire if you examined the salivary glands of this dog. The inclusions morphologically are very similar to salivary gland inclusions. I have seen Dr. Cowdry's sections showing inclusions in the tissues of dogs that were examined for another purpose. We have seen similar inclusions in the brain of a child who apparently developed a dissemination of the salivary gland virus with the production of intranuclear inclusions in the cells of many organs.

We have also recorded the experimental production of generalized visceral lesions with mouse salivary gland virus. Knowing that the salivary gland virus occasionally becomes generalized, would it not be well to consider this possibility provided the dog's salivary gland contained inclusions?

(Dr. Green.) There were, of course, routine examinations made of the salivary glands. We are well acquainted with the salivary gland inclusions from our work in examining a large variety of wild animals. There would seem to be no species of animal in which they are not found, and in some they appear to be very common. We have not attempted to carry out studies other than to note their presence. Later we shall publish these observations. From my experience in the study of encephalitis it is my opinion that the damage done to the nerve cells in these animals is sufficient to account for the symptoms.

SEASONAL VARIATION IN THE INTENSITY OF THE BRAIN REACTION OF THE ST. LOUIS ENCEPHALITIS IN MICE AND OF ENDEMIC TYPHUS IN GUINEA PIGS. R. D. Lillie, C. Armstrong (by invitation), R. E. Dyer (by invitation), and J. G. Pasternack, Washington, D. C.

Abstract. In the course of Armstrong's encephalitis studies a progressive decrease in the frequency of definitely positive brain reactions occurred, reaching a maximum in June and July, and followed by a sharp increase in August. The curve of positive reactions parallels the inverted curve of the mean monthly temperatures.

High virus dosages increased the positive reactions, but the seasonal variation remained evident in all dosage groups.

In simultaneous studies on the effect of environmental temperature 58 animals at 35°C. showed 50 per cent definitely positive reactions, 14 kept at 10°C. showed 100 per cent, and 35 at 23°C. showed 83 per cent.

Monthly grouping of 177 guinea pigs with endemic typhus revealed a similar seasonal variation in reaction intensity.

A consecutive series of 517 animals over a full year showed a high period from February to May, a low period from June to August, and a high period from September to January. The curves of the average numbers of lesions parallel the mean monthly temperature, charted inverted.

Animals at 33°C. show considerably lower average numbers of brain lesions than at 24 or 18°C.

The present indications are that environmental temperature exerts a direct influence on the intensity of the pathological reactions in the brain to these two viruses.

Discussion

(Dr. Albert B. Sabin, New York City.) I should like to ask whether only the inflammatory reactions in the mouse brains were studied, or were nerve cell lesions also noted.

(Dr. Albert E. Casey, St. Louis.) Some years ago Dr. Wade Brown noted that the transplantability of the rabbit tumor varied directly with seasonal change in temperature. And in studying blood cells we found the same variation with regard to seasonal temperature in red cells. These changes were related to seasonal variation in resistance. I am curious to know whether anything of that sort occurred here.

(Dr. Howard A. McCordock, St. Louis.) During the epidemic in St. Louis the first sharp drop in the number of cases was coincident with the return of colder weather. At that time we thought that the epidemic had burned itself out, because most of the susceptible individuals had either died of the disease or become immunized, but in view of this work it may be that the return of cold weather had something to do with the disappearance of the disease. However, I do not believe that the excessive heat during the summer of 1933 was responsible for the appearance of the disease because hot summer weather is no novelty in St. Louis, and last year we had a much higher average temperature than in 1933 without a return of the disease.

(Dr. Lillie.) In reply to Dr. Sabin, the grading given here relates entirely to the inflammatory reaction and not to the degree of destruction.

In reference to whether we have observed changes in the blood, we have

not studied blood changes, but we are carrying on investigations of other organs and we have not had time to bring this to any conclusive result before this meeting.

Dr. McCordock's remarks about the drop in the epidemic when the cold weather came on are interesting. I have speculated on it, but I do not know.

(Dr. Sabin.) In view of the fact that only the inflammatory lesions were noted, I wonder to what extent so-called "spontaneous encephalitis" of mice, unrelated to the virus injected, might have influenced the data. Among the Rockefeller Institute mice, which are bred under careful isolation, the majority of the older animals show meningoencephalitic lesions without exhibiting any clinical signs of disease.

(Dr. Lillie.) We had a very considerable number of other mice under study at the same time and they had not received the St. Louis virus. We did not see the inflammatory lesions of the brain.

THE PATHOLOGY OF GRANULOMA VENEREUM. Rigney D'Aunoy and Emmerich von Haam, New Orleans, La.

Abstract. The pathology of granuloma inguinale has been studied in a series of 294 cases observed over 5 years at the State Charity Hospital of Louisiana at New Orleans. The typical manifestations of the disease embrace nodular lesions and serpigenous ulcerations which have a tendency to spread along the moist folds of the pudendal region, healing with the formation of atrophic scars. Atypical manifestations are produced by unexplained increased fibroblastic reaction of the host leading to hypertrophic and cicatricial keloid-like lesions which must be considered active stages of the infection. Secondary infection produces serious ulcerative necrotic lesions which may severely mutilate the infected parts and give rise to sepsis and toxemia. Histopathological study of biopsy material and tissue secured from autopsy reveals that the stage of infiltration is quickly followed by the stage of granulation during which the epithelial lining of the skin or mucous membrane is perforated by a vascular granulation tissue. Donovan organisms can be demonstrated in the infected tissue during all stages of the infection. Search for a causal agent has resulted in the isolation of an organism belonging to the Klebsiella group. Inoculation of various laboratory animals with this organism has failed to incite lesions comparable to the disease in the human, although such lesions have been reported as produced by inoculation of the material derived from human cases.

AN EPIDEMIOLOGICAL APPROACH TO THE CONTROL OF TRICHINOSIS. Frank B. Queen, Denver, Colo.

Abstract. Fifty gm. portions, or less, of the diaphragms of garbage-fed and non-garbage-fed hogs grown in the Denver area were examined by the digestion method for the presence of *Trichinella spiralis*. In garbage-fed animals 15.5 per cent of the 522 examined were found to be infected. Of 193 non-garbage-fed animals but 0.5 per cent were infected.

These figures indicate that *Trichinella* infestation in hogs and hence the transmission of the disease to man can be controlled in this locality through the compulsory sterilization of all garbage fed to hogs. Studies are needed to determine the role of rats as an indirect reservoir of the disease for man.

If this source proves important, then with rat control measures added to the compulsory sterilization of garbage, trichinosis in man can be eliminated.

Discussion

(Dr. A. E. Casey, St. Louis.) Is it possible that there are factors besides the feeding of garbage, such as housing conditions and environment, which are concerned?

(Dr. Marcus W. Lyon, South Bend, Ind.) Professor H. E. Enders of Purdue told me that the examination of rats from the vicinity of slaughter houses near Lafayette showed a very high percentage of trichinosis, whereas the examination of rats living wild in fields away from slaughter houses showed a very small amount. Trichinosis is a very important subject because the hamburger stands all over the country often use a small proportion of pork mixed with the beef. In South Bend Dr. A. S. Giordano traced several cases of infestation to eating hamburger sandwiches from automobile stands along the highway. This is a very intriguing subject. The obvious way of avoiding the disease, as Dr. Queen has pointed out, is not to feed hogs anything except good food.

(Dr. E. R. Long, Philadelphia.) I might add that studies by the National Institute of Health have shown a wide prevalence of the disease in man, and the National Research Council has taken the subject under deliberation as a special problem. Two divisions of the National Research Council expect to put on a campaign in cooperation with the National Institute of Health to stimulate pathologists to go into this more thoroughly to see if figures such as Dr. Queen gave represent the present situation throughout the country. I believe the packers are very much concerned over the reports that have come out, for sales have decreased since the amount of trichinosis has been found to be so high. I wish to announce here that you will all be circularized by the Division of Medical Sciences of the National Research Council, with the request that you cooperate with the Division by submitting samples from autopsies, so that we can get a wider range of material. At present the figures from different localities vary greatly. It seems worth while to push the matter much further because the disease is evidently more widespread than used to be believed.

TOXOPLASMA INFECTION IN THE GUINEA PIG. Floyd S. Markham, Columbus, Ohio.

Abstract. Spontaneous and experimentally induced Toxoplasma encephalitis in young guinea pigs is described. Parasites were also found in the kidneys of adult stock guinea pigs. The protozoa in both adult and young animals closely resemble those observed in rabbits and mice.

Discussion

(Dr. R. D. Lillie, Washington, D. C.) In the course of examination of between 1500 and 2000 guinea pig brains in the past 8 or 10 years I ran across Encephalitozoa in 3 individual animals in which there were from 2 to 4 or 5 cysts encountered in a series of four or five transverse sections of the brain.

One of those local clumps of parasites shown on the screen in the kidney resembled Klossiella cobayae, a species often found in guinea pigs' kidneys. This species is said to form minute bodies in the vascular endothelium in other organs, but I hardly believe it to be related to the toxoplasmosis of the brain.

(Dr. Albert B. Sabin, New York City.) When this Society met in New York 2 years ago we demonstrated to the groups visiting the Rockefeller Institute studies on Toxoplasma which were isolated from a guinea pig. Proper preservation and passage at regular intervals by intracerebral injection in mice have permitted us to maintain the Toxoplasma in a pathogenic stage and to carry out many studies with them. There is considerable confusion in the literature concerning the nature of the Toxoplasma, partly because strains, as a rule, were not maintained for comparative investigations. Dr. Markham's suggestion that Toxoplasma may be related to the Encephalitozoon group of organisms, whatever their nature may be, cannot be supported by our own studies, but does point out the difficulty of making a proper diagnosis on purely morphological grounds. We are inclined to feel that widespread pathogenicity for various mammals and birds is important for the proper identification of the Toxoplasma. Our own strains are pathogenic for mice, guinea pigs, rabbits, *rhesus* monkeys, and newly-hatched and full-grown chickens. Our studies indicate that the Toxoplasma are obligate intracellular parasites which are capable of invading many types of cells, and as regards certain host-parasite relations possess many features in common with some of the filterable viruses.

Toxoplasma has been studied and reported upon in connection with certain human diseases on several occasions. In 1930 and 1931 Bland of London reported studies on their possible relation to human glandular fever (acute infectious mononucleosis), but the question naturally arose whether the Toxoplasma which were isolated had their origin in the patient's blood or in the experimental animal (rabbit). In *rhesus* monkeys we were able to show the development of protective antibodies during convalescence. By the use of the rabbit skin protection test it may perhaps prove possible to establish whether or not human infection with Toxoplasma exists.

(Dr. E. C. Rosenow, Rochester, Minn.) I would appreciate an expression from these experts on this matter. This type of infection is easily demonstrated by the presence of the microbe in the lesions. I should like an expression as to the ease with which it is found in animals that have marked perivascular lesions.

(Dr. Burton R. Rogers, Chicago.) I should like to ask Dr. Lillie whether he ever found hookworm larvae or adults in the brain.

(Dr. Markham.) Probably Dr. Sabin is much better qualified to answer the question with regard to the ease with which these protozoans are demonstrated. I have reported only an isolated observation.

(Dr. Sabin.) I should merely like to repeat that identification of such parasites purely on morphological grounds may be misleading. When we first isolated our strains we took some slides to Professor Tyzzer of the Harvard Medical School for identification. He too stressed the difficulty of morphological diagnosis and showed a slide of mouse kidney, sectioned 30 years ago, which contained parasites indistinguishable in appearance from our own.

PNEUMOCONIOSIS AND PULMONARY MALIGNANCY. Arthur J. Vorwald and (by invitation) John W. Karr, Saranac Lake, N. Y.

Abstract. The literature contains an increasing number of reports on primary carcinoma of the lung that tend to implicate inhaled dust as of etiological significance in the genesis of the newgrowth. The authors of these reports ascribe to the dust, in part at least, a carcinogenic property and point to it as one of the factors responsible for the high incidence of the tumor as encountered today. In the majority of reports, however, no inquiry has been made into the chemical and biological characteristics of the dust, and there has been no attempt to present detailed experimental and clinical evidence that the dust in question is of etiological significance and possesses carcinogenic properties. The fact that dust is a chronic irritant has been repeatedly stressed and as such has been assumed to be a predisposing factor in the development of the tumor.

A survey of pneumoconiosis cases reported in the literature, also clinical and experimental observations from the Saranac Laboratory, do not support this view. The clinical observations comprise follow-up X-ray examinations of the chests of 15,587 men from various dusty occupations. A majority of these men had had long exposures and a large proportion was over 40 years of age. Of this group, 1356, or 8.7 per cent, had demonstrable X-ray evidence of silicotic nodulation in the lungs. The incidence of primary lung carcinoma for the entire group is 0.012 per cent and for those with silicotic nodulation 0.15 per cent.

The pulmonary tissue from 179 cases of pneumoconiosis, which had developed in relation to dusty occupations in various industries throughout the country, are in the pathological museum of the Saranac Laboratory. These cases form rather a selected group, but only 3 revealed the presence of an associated primary pulmonary malignancy.

Experimental observations on different species of animals including guinea pigs, rabbits, rats, mice, chickens and cats that have inhaled dusts of varied concentration and composition for periods ranging from 1 to 3 years reveal, without exception, pulmonary foci of chronic irritation as response to the inhaled dusts. In animals with prolonged inhalation of a high concentration of silica the foci were nodular in character and made up of hyaline fibrotic tissue not unlike those in clinical cases of silicosis. Hyperplasia of the epithelium lining the major bronchial tree was seldom observed. The terminal air passages adjacent to and within the foci of chronic irritation were often lined with a flat, compact single layer of epithelial cells. Even under these conditions, the incidence of primary lung tumor was practically nil. Only two small, well localized tumors were found in the lungs of 3338 animals that had been exposed to dust.

Our evidence is significant enough to indicate that dust inhalation seldom, if ever, stimulates a reaction that terminates in a primary pulmonary malignancy.

Discussion

(Dr. Samuel R. Haythorn, Pittsburgh.) If there is any connection between the inhalation of dust, smoke and primary tumors of the lung, it probably is in connection with the tar content of soft coal smoke. With this idea in mind

we placed mice in soft coal chambers in an experiment that I am going to describe later in the day. So far we have been very fortunate, for we have had only one lung tumor, and it occurred in a mouse in the control cage where it had breathed nothing but washed filtered air. There was no chance to draw the conclusion that it was other than accidental.

(Dr. Norbert Enzer, Milwaukee.) I should like to emphasize one point made by Dr. Vorwald with reference to the presence or absence of epithelial hyperplasia in the bronchi, for that would be an indication of the degree of reaction of the epithelium to the irritant. In 125 postmortem examinations of individuals with varying degrees of silicosis the bronchial mucosa failed to show any evidence of polypoid formation or atypical papillary formation, and in only one instance was there a finding of squamous cell metaplasia, and that occurred in a tuberculous bronchiectatic cavity. In the entire 125 cases not a single instance of bronchiogenic carcinoma was noted.

(Dr. H. Gideon Wells, Chicago.) A great deal depends not only on the irritant but on what is being irritated. Dr. Slye has one strain of mice in which almost 100 per cent have developed lung tumors merely by breathing the pure air of Chicago, whereas many thousands in other strains have gone on and died of old age with no member of those strains ever showing anything of the sort, so that we always have to consider two factors. That is the difficulty in all such experimental work, unless you know the nature of the animal with which you are working.

(Dr. M. J. Shear, Boston.) I do not know whether the air in Chicago is any purer than that in New York, but the dust in the air in New York has a significant amount of tar in it. Dust from the air in New York has been collected and has been found to contain a carbon disulfide soluble fraction; this air dust tar is being tested in our laboratory for carcinogenic activity, using albino mice.

(Dr. Wells.) Probably there is more here because we use a greater percentage of soft coal.

(Dr. Vorwald.) We realize that tar has carcinogenic properties. A good many animals have been subjected to soft coal dust. Some have even lived in the soft coal mines where the dust was generated. These were included in the report.

STUDIES IN CARCINOGENESIS. V. METHYL DERIVATIVES OF 1,2-BENZANTHRACENE. M. J. Shear (by invitation), Boston, Mass.

Abstract. Previous collaborative studies showed that cholanthrene had about the same carcinogenic potency as methylcholanthrene and that therefore the methyl group of methylcholanthrene is not essential for high activity. It is now found that the 5-membered ring characteristic of the cholanthrenes is not required for high potency.

Methyl derivatives of 1,2-benzanthracene, synthesized by Prof. L. F. Fieser and his collaborators, were injected subcutaneously into Strain A mice. With 5,10-dimethyl-1,2-benzanthracene, ulceration began after 2 weeks and became extensive during the 2nd month. In 2½ months tumors were noted in 7 of 36 mice; in 4 months 20 tumors were obtained. One of these tumors was discontinued after being transplanted twice; another tumor is now in its fourth generation.

While the 5-methyl derivative produced no tumors in 20 mice in 2 months, the 10-methyl derivative produced 8 tumors in 20 mice in the same period. The former compound gave a total of only 6 tumors in 5 months, whereas the latter produced 15 tumors in 4 months. Three of the tumors produced by the 10-methyl compound were successfully transplanted; 2 of them are now in their fourth generation. The 10-methyl compound produced extensive ulceration after 1 month.

The 7-methyl, the 9-methyl, and the 5,9-dimethyl derivatives of 1,2-benzanthracene are also being tested. Of these, only the last one has displayed biological activity during the first few months.

EXPERIMENTAL PRODUCTION OF ANILINE TUMORS OF THE BLADDER IN DOGS. W. C. Hueper and (by invitation) H. D. Wolfe, Wilmington, Del.

Abstract. The investigation was undertaken to obtain experimental proof for the existence of causative interrelations between prolonged exposure to certain aromatic amines and the development of tumors in the urinary bladder. Sixteen female dogs were treated since May, 1935, with daily subcutaneous injections of 12 to 15 mg. of commercial betanaphthylamine. As there was no evidence of tumor formation in the bladder after 1 year of continued treatment 10 dogs of this series received in addition to the injections daily 150 to 450 mg. of betanaphthylamine by mouth. In January, 1937, 2 dogs of the oral group showed diffuse papillomatosis of the urinary bladder on cystoscopic examination. Two months later single papillomas were found in the bladders of 2 additional dogs, while a 5th dog had a suspicious lesion. The tumors were mainly located in the dome and the anterior wall of the bladder, that is, in the dependent parts. Biopsies taken through the cystoscope showed on microscopic examination papillary lesions of benign and malignant character resembling closely, in regard to the morphology of the tumor parenchyma and stroma, those occurring in men after exposure to aromatic amines. One dog died 3 days after cystectomy and uretero-utero-anastomosis and had in addition to numerous sessile and pedunculated neoplasms of the bladder a marked nodular cirrhosis of the liver. Lactating breasts were present in 7 of the 16 dogs on external examination in March, 1937.

Discussion

(Dr. Stanley Reimann, Philadelphia.) I should like to know what the 2 per cent of impurities are.

(Dr. Virgil H. Moon, Philadelphia.) What was the character and distribution of the cirrhotic changes seen in the liver?

(Dr. Hueper.) The betanaphthylamine is 98 per cent pure and contains almost 1 per cent of beta-betadinaphthylamine, which is structurally very closely related to the 3,4,5,6-dibenzcarbazone, which according to Cook produces epitheliomas of the skin of the mouse. We do not know at the present time whether massive doses of betanaphthylamine produce that effect, or whether a metabolite of betanaphthylamine, according to Cook, 3,4,5,6-dibenzcarbazone, or an impurity in the betanaphthyalmine, such as beta-betadinaphthylamine, is the carcinogenic agent.

So far as the distribution of the cirrhotic changes goes, the cirrhotic changes

were most marked in the right liver lobes, and they appeared as indistinctly outlined white-brown nodules projecting above the surface. Histologically they appeared as rather diffuse proliferations of liver cells, in places invading degenerative parts of the original liver structure.

Histological examination of the breast showed a typical lactating breast.

CARCINOMA IN FROGS AND THE PROBABLE ETIOLOGICAL RELATION TO A VIRUS. Balduin Lucké, Philadelphia, Pa.

Abstract. For the experimental study of cancer, warm-blooded animals such as rodents and fowls have hitherto been used. Recently it has been shown that a cold-blooded and more primitive animal, the leopard frog (*Rana pipiens*) is commonly affected with a carcinoma, and that this tumor is suitable for experimental studies (*Am. J. Cancer,* 1934, **20**, 352, and 1934, **22**, 326). It is a typical neoplasm, and it has the particular interest in that its cell nuclei frequently contain acidophilic inclusion bodies of a type characteristic of virus activity. Four hundred and twenty-two cases of the tumor have been examined; of these 364 are spontaneous growths and 58 are from frogs inoculated with tumor material.

In the present paper further observations on metastasis are recorded, and evidence is presented that the frog carcinoma is probably caused by a virus.

Metastasis: In our earlier studies but few examples of dissemination (3 among 276 cases of tumor) were encountered; this apparent rarity of metastasis seemed to be a fundamental difference between the frog carcinoma and structurally similar tumors of man and other warm-blooded animals. That no such difference exists is proved by our more recent findings of 17 cases of neoplastic dissemination among the 146 frog tumors examined since the publication of the previous report. Commonly, the metastatic tumors are multiple and involve more than one organ, the liver being one most often the site of the secondary tumors. Tumor emboli lying within intrahepatic branches of the renal portal veins in several of these cases suggest not only the route of spread, but make it certain that we are dealing with true cellular metastasis and not with secondary growths incited by dissemination of a causal agent. The pancreas, lung, various parts of the gut tract and the orbit are other sites to which the tumor has extended, also, in all probability, by way of the blood. On the other hand, some secondary tumors lying in the urinary bladder and the ovarian stroma may represent direct implantation of fragments detached from the primary growth rather than transport by the bloodstream.

Transmission Experiments: Material from 45 different tumors has been inoculated by various routes (intramuscularly, into the lymph sacs, the pleuroperitoneal cavity, and intracranially) into a total of over 800 frogs of the same species and from the same general locality as the tumor-bearing frogs. A somewhat larger series has been kept under identical conditions as controls. In 34 of the experimental groups living tumor fragments or cell suspensions were used; in 10 tumor desiccates, and in 1 group glycerinated tumor material. The complete results are not as yet available since some of the animals are still alive, but the majority have been examined. While in some frogs the transplanted fragments persisted for several months and at the time of examination presented evidence of cell multiplication (mitoses), most fragments soon retrogressed, and no growths of significant size were observed in any of

the groups at the site of inoculation. However, a considerable number of the inoculated frogs developed tumors in the kidney. These renal tumors were morphologically identical with the spontaneous carcinomas; their incidence increased progressively with the length of period of survival after inoculation. Thus, in frogs that died or were killed within 3 months after inoculation, the incidence of the renal tumors was approximately the same as in the control groups, *i.e.* 2 to 3 per cent. In frogs that survived for from 4 to 6 months the frequency of renal tumors rose to 9 per cent, and to double this figure in the groups surviving for more than 6 months. No difference was observed between the groups that were inoculated with living material and those that had received desiccated or glycerinated tumors; in both series kidney tumors occurred with approximately the same frequency and after the same interval following inoculation. The fact that tumors developed as readily in animals inoculated with tumor desiccates as with living cells, and that these tumors developed not at the point of inoculation but in the tissue in which they naturally occur argues, we believe, for the existence of an organ-specific virus.

DEVELOPMENT OF ODONTOMAS IN RATS FOLLOWING A PROLONGED CHRONIC VITAMIN A DEFICIENCY. Caspar G. Burn and (by invitation) Alvin U. Orten and Arthur H. Smith, New Haven, Conn.

Abstract. A mild chronic vitamin A deficiency has been produced in albino rats by administering by mouth minimum quantities of a standardized dose of cod liver oil. The amount of vitamin A given daily was determined for each individual rat by the body weight, appearance of eyes, snuffles and vaginal smears. The average daily dose of vitamin required from 0.7 to 8.0 International Units. The most striking gross change in the rats surviving from 80 to 365 days consisted of loss in color of the incisor teeth, distortion of shape, such as twisting, transverse and longitudinal ridging, and eventual exfoliation of the erupted portion and tumor formation. Histologically some of the rats showed changes compatible with those found in a complete vitamin A deficiency. Tumor growths (odontomas) developed in over 60 per cent of the rats surviving 365 days. A few rats developed supernumerary incisor teeth. The tumor consisted chiefly of spindle shaped cells similar to the embryonic cells of the pulp tissue. Inclusions of odontoblasts and epithelial cells were distributed throughout the tumor proliferation. Imperfect forms of germinal tooth centers and osteodentinal structures were frequent. The molar teeth of the rats did not show tumor growths.

Discussion

(Dr. S. Burt Wolbach, Boston.) It is needless to say I am tremendously interested in the consequences of this very prolonged partial vitamin deficiency. From what I have seen myself I am not surprised that these results have been obtained. I doubt very much whether the majority of us here would accept the newformations as tumors in the sense in which we usually think of tumors, but nevertheless I think they may very well be. The one thought I should like to leave with you is that after all a vitamin deficiency deprives a cell of something necessary, and according to recent progress in biological chemistry it seems very probable that the removal of a vitamin

interferes with some one particular type of biochemical system. When we can do this to cells and leave the cells under conditions that enable them to survive and to multiply, we have produced very extraordinary conditions. I think this work represents a much greater progress in that line than we have made in Boston.

EXPERIMENTAL CARCINOMA OF THE PROSTATE. Robert A. Moore and (by invitation) Robert H. Melchionna, New York City.

Abstract. The paper reports a study of the effects of injection of a carcinogenic chemical, 1:2 benzpyrine, on the prostate, and the relation of the testis to the production and course of the tumors produced.

White rats of unknown genetic constitution, but derived from one breeding colony and maintained on an adequate diet, were used. The animals were about 150 days of age at the time of first procedure except those designated as senile, which were over 500 days of age. Castration was done through a single abdominal incision. The 1:2 benzpyrine in 5 per cent concentration was dissolved in lard at temperatures not above 100°C. and allowed to congeal. One-tenth of a cc. was injected through a fine needle into each anterior lobe of the prostate exposed by a midline incision. Lard alone was injected into control animals. The tissues were fixed in Bouin's fluid and paraffin sections stained with hematoxylin and eosin.

There was a total of 18 rats in which no additional procedure was introduced. These animals died or were killed from 110 to 210 days after a single injection of benzpyrine. Seventy-two per cent showed carcinoma and 5 per cent also sarcoma. In 20 animals subjected to castration at the same time that the benzpyrine was injected and autopsied 70 to 210 days afterward, the incidence of carcinoma was 65 per cent and of sarcoma also 5 per cent. Twelve senile rats were injected and observed up to 352 days after the experimental procedure. In 7 the period of observation was comparable with the above two groups and the incidence of carcinoma was 86 per cent with no example of sarcoma. The remaining 5 were autopsied after 250 to 352 days and all showed carcinoma and 2 developed sarcomas.

Six adult animals on the 178th day after injection were subjected to a laparotomy, a small biopsy specimen of the prostate taken and the testes removed. Thirty-five days later the animals were killed and the character of the tumor in the biopsy and autopsy sections compared. There was no essential difference. Four animals were castrated and injected with benzpirine and after the 86th day given a daily injection of 0.25 mg. of the propionic acid ester of testosterone (Oreton, Schering). One animal died after 48 daily injections and the other 3 were killed after 62 injections. All showed well developed carcinoma and the latter 3 definite sarcoma. All control animals were negative.

In a study of the histogenesis of the tumors it seems probable that the action of benzpirene is first to produce squamous metaplasia of the normal columnar epithelium and then malignant proliferation of the metaplastic cells. The sarcomas originate in the stroma immediately adjacent to a benzpirine cyst and usually, but not exclusively, in cysts that are not lined by epithelium. It is probable that this tumor is a leiomyosarcoma.

Discussion

(Dr. Marion, Chicago.) Has lard alone been used as a control?

(Dr. Howard T. Karsner, Cleveland.) In order to clarify the matter I want to ask Dr. Moore two questions. One is as to whether or not sarcoma and carcinoma occur simultaneously in the same prostate, and the other is as to what evidence he can bring forward to support the contention that the sarcoma develops from smooth muscle cells.

(Dr. Moore.) I should have mentioned that we have 20 animals of the intact type and 15 castrates that were injected with lard alone. There was in some a foreign body type of reaction and slight fibrosis. There was never any evidence of metaplasia or any evidence of neoplastic proliferation of the connective tissue.

All the sarcomas we have observed have been in glands that also showed carcinoma. Sarcoma has never occurred alone.

As evidence for the smooth muscle, we have given a great deal of attention to this because in some areas there was evidence that the tumor might be derived from the skeletal muscle in front of the prostate, about the bladder neck. There was also histological evidence that it was growing from the adventitial cells about the blood vessels. We have come to the tentative conclusion that it is derived from the smooth muscle on the basis of differential stains; it stains as smooth muscle fibers with Van Gieson's, Mallory's and Masson's stains, and there was no evidence of reticulum formation in among the tumor cells.

GYNECOMASTIA ASSOCIATED WITH INTERSTITIAL CELL TUMOR OF THE TESTIS.
John W. Budd, Los Angeles, Calif.

Abstract. A 42 year old white male had suffered from enlarged and painful breasts for 5 months and impotence for 2 months. Examination revealed a swelling in the right testis which upon exploration was proved to be an encapsulated tumor 25 cm. in diameter. Microscopically the tumor cells were identified as interstitial cells. Assay of the urine and tumor tissue was negative for the presence of prolan. Twenty mouse units of estrin were found in 1.73 gm. of tumor. In the 3 months following operation there has been a material reduction in the size of the breasts and the impotence has been partially overcome.

Discussion

(Dr. E. T. Bell, Minneapolis.) I think there is a serious question here whether this is an interstitial cell tumor or not. Its morphology is very much like that of the ordinary embryomas of the testis, and its hormonal effect is just the opposite of what we would expect from an interstitial cell tumor. Interstitial cell tumors secrete the male hormone, and I do not see how you can connect gynecomastia with the male hormone. My impression of the tumor is that it is an ordinary embryoma of the testis.

(Dr. H. Edward MacMahon, Boston.) I should like to ask about the state of the tissue in the testis surrounding the tumor as well as the condition of the other testis. Dr. Budd told us the patient was impotent for 2 months.

Gynecomastia associated with impotence is seen in cases of fibrosis and atrophy of the testis in the absence of any tumor.

(Dr. Paul Klemperer, New York City.) I saw one tumor I thought was an interstitial cell tumor of the testis, and there was one striking feature — marked pigmentation. In the tumor presented the gross description was yellowish white. Was there any pigment visible microscopically?

(Dr. Virgil H. Cornell, New York City.) May I ask what the effect of the operation was on the gynecomastia?

(Dr. Victor Lespinasse, Chicago.) What about the hormones in the urine?

(Dr. Budd.) Last year Dr. Bell and associates reported a case of interstitial cell tumor in a boy associated with the macrogenitosomia syndrome, one of 3 cases in the literature. I think the physiological effect on an adult from the excessive hormone secretion might be nil or of a different nature. I am glad of Dr. Bell's criticism, and I should like to exchange slides with him.

Dr. MacMahon asked about the other testis. The other testis is normal by palpation; the patient has been seen several times since the operation and it still remains normal. The testis removed with the tumor showed rather marked atrophy; spermatogenesis was greatly reduced, more than the mere mechanical pressure of the tumor would account for.

Dr. Klemperer asked about the pigmentation. The color of the tumor was not so striking as in some cases of interstitial cell tumors that have been described. There was a definite yellowish color, but pigment microscopically was not found. This tissue was fixed in Zenker's, and I have noticed that Zenker's fixative does have some tendency to dilute pigmentation in the cells, and perhaps that would obscure a minor amount of pigment present.

Dr. Cornell asked about the effect of the operation on the gynecomastia. In the 3 months since the operation there has been a definite reduction in the size of the breasts. They are still larger than the breasts before the onset of illness, but there is a very definite clinical reduction in the size of the breasts.

In regard to the question about the hormones, this patient's urine previous to operation was assayed for prolan and none was demonstrated. The tumor tissue was assayed for prolan and none was demonstrated. The acetone extract of 1.73 gm. of tumor tissue revealed the presence of 20 mouse units of estrin. The hormone which we are interested in is the male sex hormone. The identity of the tumor was not appreciated until after the permanent sections were viewed, so that the matter of assaying for male sex hormones was delayed, and this is being conducted at the present time. I am sorry I do not have the reports to present at this time.

INTRINSIC FACTORS IN THE ETIOLOGY OF NEOPLASMS. Carl V. Weller, Ann Arbor, Mich.

Abstract. In the etiology of neoplasms both intrinsic and extrinsic factors have a share. The part played by intrinsic factor may be evaluated, so far as our present knowledge permits, by both clinical and experimental studies. From the clinical side the family histories of carcinoma patients, the study of families with a high incidence of carcinoma, the occurrence of multiple neoplasms in the same patient in excess of chance distribution, the modes of inheritance of particular neoplasms, and the occurrence of simultaneous and/or

symmetrical similar neoplasm in monochorionic twins have been the chief lines of approach. Each of these must receive critical evaluation. From the experimental side the breeding of strains of laboratory animals that develop spontaneous carcinomas to a high degree, or not at all, has been followed by more precise methods of investigation according to the technic of present day genetics. Into animals of tumor-producing and non-tumor-producing strains, and into their hybrid offspring, neoplasms have been transplanted and the results analyzed. Furthermore, the response of animals of varying constitution in respect to carcinoma production, and to known extrinsic carcinogenic agents reveals varying levels of resistance to such agents. From these many lines of approach it is evident that intrinsic factors enter at two levels. For the development of any neoplasm the intrinsic factor of potentiality of cellular multiplication and growth must be present. In addition there is abundant evidence that predisposition to neoplasia depends in varying degrees and through varying mechanisms for different neoplasms on intrinsic, and sometimes inheritable factors. Some of these are effective through the production of potentially preblastomatoid somatic variations. From the practical side, and for any particular human being, the importance of carcinoma in his family history must be interpreted with due regard to carcinoma type, anatomical distribution, and totality of carcinoma incidence.

RESPONSES TO CARCINOGENIC CHEMICALS ANTECEDENT TO TUMOR FORMATION. S. Burt Wolbach, Boston, Mass.

Abstract. For studies of connective tissue responses cylindrical pellets composed of 5 per cent 1,2,5,6-dibenzanthracene and 95 per cent cholesterol were introduced subcutaneously in mice.

For studies on epidermal responses mice were painted on the skin of the back with solutions of 3,4-benzpirine in benzene. The responses in the liver to 2-amino-5-azotoluol (o-amido-azotoluol) were studied in rats and in mice. With rats, the chemical was introduced with the food (Yoshida). With mice, Shear's method of injecting the chemical suspended in glycerin into the subcutaneous tissues was used.

The effects of these three chemicals were followed histologically. All three proved to be destructive agents. With all three the sequences indicate that constant reparative processes are maintained. These reparative processes involve repair of damage to individual cells, as well as replacement of cells by division of adjacent cells. With all three agents a period of hyperplasia occurs and there is evidence that this takes place only after some degree of resistance has been acquired by the cells concerned to the agent employed. This is particularly striking in the case of the connective tissues about pellets containing dibenzanthracene. In all three experiments reparative responses on the part of resistant cells are the presumable explanation of hyperplasia, strikingly evident in islands of regeneration in the liver in the o-amido-azotoluol experiments and in the behavior of hair follicles in skin-painting experiments with benzpirine. Even after the stage of hyperplasia is reached, evidences of the injurious effects of the chemicals on the cells continue to appear. Maintained reparative responses in regions of hyperplasia seem to be the stage preceding the development of true tumor.

No evidence was found that could be used to support a theory that any

one of the chemicals employed owes its carcinogenic property to direct stimulation of cell growth.

RELATION OF FILTERABLE AGENTS TO TUMOR FORMATION. Peyton Rous, New York City.

Abstract. Filterable agents are responsible for various tumors of the domestic fowl and for a skin papilloma of the rabbit, which frequently becomes carcinomatous. The renal tumors of the leopard frog are probably due to such a cause.

The agent producing the rabbit growths is characteristically a virus. Several reasons have been advanced for supposing the chicken tumor agents to be of a different sort, but with increasing knowledge it has become plain that they too fall into the virus class. The view that they are lipids is not tenable.

Under ordinary conditions cancer does not develop from the rabbit papillomas consequent on cutaneous inoculation until they have proliferated for several months. If, however, the virus is thrown into the blood stream of animals that have been tarred on the ears for a brief period, it localizes in the disordered skin and primary carcinomas arise there as well as papillomas. The way in which the malignancy comes about has still to be elucidated.

The viruses responsible for the chicken and rabbit tumors act as the immediate, directly inciting causes of neoplasms. The other "carcinogenic" agents thus far studied appear to do no more than prepare the tissue for the action of some immediate cause of nature still unknown.

SEX HORMONES AND THEIR RELATION TO TUMORS. Leo Loeb and (by invitation), E. L. Burns, V. Suntzeff, and Marian Moskop, St. Louis, Ill.

Abstract. An ovarian hormone, estrin, acting in cooperation with hereditary factors ($H \times S = C$), causes the transformation of normal mammary gland tissue into carcinomatous tissue. This change takes place step by step and is the result of a summation of growth stimulations. In addition, the growth-inducing stimuli acting on the mammary gland during pregnancy intensify the carcinomatous transformation. Estrin induces also in vagina and cervix the formation of epithelial processes reaching into the connective tissue and progressing to the production of carcinomatous or carcinoma-like lesions in a certain number of cases. This process also takes place step by step. Estrin produces therefore carcinomatous or carcinoma-like changes in tissues in which it normally initiates specific growth processes. An increase in the amount of tissue, which occurs in response to hormonal stimulation, is not the essential or a necessary condition in the carcinomatous transformation, but it represents an associated factor produced by the same condition which is responsible for the development of carcinoma. Also, sarcoma may develop in the injected mice, but this is the result of non-specific growth stimulations.

Enlargement of the anterior pituitary gland which follows long continued estrin injections is not directly connected with the development of mammary gland carcinoma, inasmuch as this enlargement may occur most frequently in low tumor rate strains. However, it is possible to induce carcinoma formation in the mammary gland in suitable mice through multiple transplantations of anterior pituitary glands.

In high and low tumor rate strains the effects of estrin injections on the mammary glands are similar during the first 3, 4 or 5 months of injections; only subsequently the response of this tissue begins to differ in different strains. The hereditary differences in the tendency to the formation of carcinoma depend probably on differences in the mode of response of a certain tissue in different individuals or strains to long continued stimulation. The hereditary conditions underlying the proliferative changes in the mammary gland and in vagina and cervix are not the same. These differences do not depend, therefore, on differences in the rapidity of elimination of estrin from the organism.

The change of whole mammary gland tubules and acini into carcinomatous tissue, which may occur simultaneously in various parts of this organ, cannot be due to somatic mutations, nor does it depend on preceding inflammatory conditions. It is due to the cumulative action of growth stimuli of various kinds in association with hereditary factors. The action of carcinogenic hydrocarbons is in principle not different from that of other carcinogenic factors; they all seem to act through long continued growth stimulations. It may be assumed that the carcinogenic effect of hormones and other growth-inducing agents depends on an intracellular production of growth-stimulating substances which are constantly newly formed by processes similar to autocatalysis, or which are otherwise self perpetuating. It may be furthermore assumed that under certain conditions these substances are separable from the cells in which they developed and that they may then induce the production of similar growth substances in analogous tissues of other animals.

The recent experiments of Peyton Rous concerning the action of a virus in epidermal carcinoma in the rabbit suggest that possibly also in hormonal carcinomas extrinsic viruses may play a role. However, this question must be left for further investigation.

HISTOPATHOLOGICAL CHANGES IN MARKED SWELLING OF THE BRAIN. Kornel L. Terplan, Buffalo, N. Y.

Abstract. For several years systematic histological studies of the central nervous system were undertaken in cases that showed a distinct swelling of the brain postmortem. In all of these cases certain clinical symptoms such as stupor, unconsciousness, twitching, convulsions, and usually severe coma, had pointed to grave functional disorders of the brain. These studies started with a most impressive case of fatal insulin shock in a 16 year old boy with excessive swelling of the brain and the spinal cord. Histologically in this case marked structural changes in the cortex with almost complete disappearance of certain ganglion cell layers were found.

In order to get more information about the etiological and also perhaps the pathogenetic factors involved in this destructive process a large series of cases was examined, all of which showed distinct swelling of the central nervous system at autopsy.

This presentation includes the findings in the following conditions: 2 cases of insulin shock; 3 cases of acute encephalitis in children, in 1 of which severe hypoglycemia was produced by faulty administration of insulin; 1 case of oleum Chenopodium poisoning; 1 case of second degree burns with a past history of whooping cough; and 1 case of cerebellar cyst with diffuse swelling

of the brain. In this last instance, previous to operation, under the effect of avertin, respiration had ceased temporarily.

The histological changes found in the cortex of all of these cases suggest, in spite of the different causative factors, a similar pathogenetic mechanism. These changes, however, were not found in uremia, diabetic coma, inner hydrocephalus, purulent meningitis, and a considerable number of other conditions that were used as controls.

At postmortem examination, which, as a rule, was performed within the first 6 hours after death, the brain showed a marked swelling with distention of the dura and completely flattened gyri. Very little spinal fluid was seen within the ventricles and hardly any in the subarachnoid spaces. The weight of the brain was always distinctly increased.

The histological changes in many of these cases appeared to be of such marked intensity that they should be readily detected without any specific knowledge of the finer nervous structures. They consisted of distinct swelling of the entire cortex with marked destruction of the normal architecture. The nerve cells, especially in the third and fifth layers, were almost entirely destroyed, which made recognition of the typical layering very difficult. Where the ganglion cells could still be recognized colliquation necrosis or ischemic necrosis was present; other cells appeared as so-called ghost cells. The capillaries showed extreme injection and swelling of the endothelial cells, and the entire capillary network was conspicuous. In addition, there was slight or moderate proliferation of neuroglia cells, some of which showed severe regressive changes. These changes were especially marked in 1 case of insulin shock and in that of the cerebellar cyst where breathing had ceased temporarily under the effect of avertin. The frontal lobe, the island of Reil, and the hippocampal gyri, including Ammon's horn, were more markedly involved than the central and occipital areas. In certain instances, especially in the case of oleum Chenopodium poisoning, there was an absence of nerve cells, practically restricted to the third layer. Only in 1 case was it possible to study the later stages of this severe destruction. In this case the patient died 11 days after a state of complete unconsciousness from anesthetics given during an operation for fibroids. Respiration in this case had ceased for a short time. The nerve cells in the third layer were entirely replaced by a dense network of proliferating capillaries. There was also considerable demyelinization in the cortex, especially in the frontal and occipital area.

Changes similar to those found in the cases of insulin shock have been produced in the last 2 years in rabbits and dogs in experimental insulin shock by Grayzel, and Stief and Tokay. Grayzel felt that the severity of the cortical changes was parallel to the number and severity of the convulsions seen in these animals. The convulsive mechanism has been stressed, particularly by Spielmeyer in his pathogenetic studies, with regard to the changes in Ammon's horn in genuine epilepsy. To my knowledge, however, such extensive changes in different cortical areas have not been observed in cases with death in acute epileptic seizures. In 2 of our cases presented here, at no time were convulsions observed. Apparently the pathogenetic factors include severe nutritional disturbances in the widest sense, such as lack of blood supply or lack of oxygen alone, or, especially in insulin shock, the incapacity of the tissues to utilize the oxygen present in normal amount in the blood. From the control material we have examined so far we do not feel that mechanical factors

alone will produce this type of cortical destruction. The findings here presented again prove the marked sensitivity of the third layer in the cerebral cortex, and of the valleys between the gyri where all the changes appeared especially intense.

Discussion

(Dr. Shields Warren, Boston.) In the few cases of insulin shock which I have had an opportunity of seeing at autopsy I was rather more impressed by the degree of cerebral edema immediately beneath the ependyma in both the hemispheres and the midbrain than I was by that in the cortical layer. I wonder what Dr. Terplan's observations have been in regard to that.

(Dr. Sheldon A. Jacobson, New York City.) I should like Dr. Terplan to tell us something about these brains from the mechanical point of view. It is rather difficult to understand just how swelling of the brain as a whole could take place to a great extent. I understand in these cases there must have been a diminution in the amount of cerebrospinal fluid. In general, I think the diagnosis of edema of the brain is often made when one should say wetness of the brain. Certainly that is true in most of the brains called edematous which I have seen. Wetness might represent a change from a gel to a sol without any change in the fluid content. Schmorl made a study of the intravertebral discs and found that while there was an enormous difference in wetness, on chemical analysis there was no change in the water content. It is easy to understand how brain tissue might swell up when there is an abscess with destruction; otherwise it is hard to understand.

(Dr. Virgil H. Moon, Philadelphia.) I should like to ask Dr. Terplan if records were made of the visceral changes in parts other than the brain; whether congestion and edema of the lungs, the gastro-intestinal mucosa and the liver and kidneys were features in these cases. I have had no opportunity to make postmortem examinations on cases of insulin shock, either clinically or experimentally produced, but I have the feeling that changes of a circulatory character would be found similar to those that accompany shock originating otherwise.

(Dr. Terplan.) In reply to Dr. Warren, in all of the cases presented the entire brain was examined, which included, of course, the periventricular structures. Marked edema or, as I prefer to say, distinct swelling of the brain tissue, was present throughout, especially around the ventricles which, as I have mentioned, were distinctly compressed by the swollen brain. However, severe destructive lesions which were so conspicuous in the cerebral cortex were not seen in the basal ganglia around the ventricles. Only the striatum showed slight defects of nerve cells.

In regard to Dr. Jacobson's remarks, the term "swelling of the brain" was used in the sense of Reichhardt. In these cases the dura mater was markedly distended by the swollen brain, the gyri were flattened, and the sulci entirely obscured, as were the basal cisternae. There was a minimal amount of spinal fluid in the ventricles. The absence of spinal fluid in the basal cisternae, and the distention of the spinal dura by the swollen cord in the cases of insulin shock were very impressive. Of course it is mandatory to perform the postmortem examination very shortly after death to observe true swelling of the brain. We know that the amount of spinal fluid decreases about 6 hours after death by 25 per cent, and about 6 hours later by almost 50 per

cent. There exists, however, a distinct diffuse brain swelling outside of collateral edema around abscesses or other lesions. The term "disturbed colloid balance" has been used as an expression of true swelling of the brain by Schlueter and Seifert (London) who claim that the freezing point of the white matter in these cases is decidedly lowered. The markedly diminished amount of spinal fluid seems to me the most important diagnostic criterion for true swelling of the brain.

In answer to Dr. Moon, the liver in the cases of insulin shock was carefully examined as we were especially interested in the glycogen findings. Glycogen was entirely absent, and neither grossly nor microscopically were changes of distinct edema noted. In the lungs especially, only recent petechial hemorrhages in the pleurae and slight hypostasis were seen, but I do not recall any impressive edema in the viscera outside of the brain.

A Practical Classification for Hepatic Cirrhosis. Virgil H. Moon, Philadelphia, Pa.

Abstract. Cirrhosis is best defined as *chronic diffuse hepatitis.* An etiological classification is impracticable, for the causes of hepatitis are various and some are obscure. Different cirrhotogenic agents may produce the same type of disease. Hence, a satisfactory classification must be based on morphological characteristics, *i.e.* on the nature and distribution of the process.

Morphological studies on portal cirrhosis indicate that its distinguishing feature is destruction of lobular pattern. There are no longer *lobules* but only *nodules* of hepatic cells. This criterion has been used satisfactorily in making differentiations. The following classification is based on it.

Chronic Hepatitis — Cirrhosis

1. With Obliteration of Lobular Pattern

(*a*) Portal cirrhosis (Laënnec's)
(*b*) With extensive pigmentation — hemochromatosis

2. Without Obliteration of Lobular Pattern

(*a*) With perilobular fibrosis
Perilobular (monolobular) cirrhosis
(*b*) With biliary obstruction
Biliary (obstructive) cirrhosis
(*c*) With fibrosis about central veins
Central cirrhosis (chronic passive congestion)
(*d*) With irregular varying fibrosis
(1) With evidence of active degeneration and repair
Early portal (Hanot's, hypertrophic)
(2) Fibrosis of irregular distribution
Atypical cirrhosis

Discussion

(Dr. Paul Klemperer, New York City.) Dr. Moon, if I understood you correctly, you would not make any difference in your classification between the so-called toxic cirrhosis and the complex group of Laënnec's cirrhosis.

I wonder if I am correct in that, because I do feel that we should make a very definite distinction between the so-called toxic cirrhosis, which is primarily due to a destruction of the parenchyma and leaves the framework intact, and Laënnec's cirrhosis. Comparing the anatomical and histological picture in toxic cirrhosis with that in Laënnec's cirrhosis, in which I realize there is also parenchymal destruction, I believe the parenchymal destruction is not primary in Laënnec's cirrhosis. I wonder if we should define toxic cirrhosis as diffuse chronic hepatitis; the inflammatory factor in toxic cirrhosis is secondary and not primary. In regard to the so-called Hanot's cirrhosis, I think Hanot had something very definite in mind; certainly one group of his cases was an intrahepatic biliary obstructive cirrhosis. It is the obstruction of the intrahepatic bile ducts which leads to a biliary obstructive cirrhosis.

(Dr. Moon.) In answer to Dr. Klemperer's question, this classification is based on morphology, rather than on etiology. The term "toxic" implies an etiological agent. There are several kinds of cirrhosis which can be produced by toxic agents, and that is one reason for confusion in classification. I should prefer to speak of a portal cirrhosis due to arsenicals, a portal cirrhosis due to streptococcus infection, a portal cirrhosis due to carbon tetrachloride, and so on, when the etiological agent is known.

Regarding that group of cases classed as atypical, I recall that our esteemed colleague, Dr. Mallory, surveyed 10,000 autopsies and found 590 cases of cirrhosis. Of these he found that 18.5 per cent were not classifiable. Cirrhoses having the irregular type of distribution illustrated in the last two slides do not conform to any classification, nor do they produce characteristic symptomatology. The only types that produce definite symptoms are the portal cirrhosis, biliary cirrhosis with obstruction, and perhaps Hanot's cirrhosis.

Regarding Hanot's cirrhosis, the first International Congress for Geographic Pathology had as its subject Cirrhosis. Hanot's was one of the types considered. Reports were presented from 26 countries. It was interesting to note that from France, the country of Hanot's origin, there was only 1 case reported. There was not 1 case from the United States. Hanot's cirrhosis is a conception that has no definable entity. I prefer to classify cirrhoses that are not characteristic morphologically as *atypical cirrhosis.*

THE INTERRELATION OF OSTEOGENIC TUMORS. Sheldon A. Jacobson (by invitation), New York City.

Abstract. An attempt is made to unify our concepts of the tumors of bone, and particularly of those that are not self-limited. The solitary osteomas and osteochondromas represent, of course, the benign form. It has been pointed out that the level of physiological activity of bone varies from its height in the metaphyses through the epiphyses and round (carpal and tarsal) bones to its lowest point in the diaphyses. This is exemplified by their differential growth rates and reactivity to such injuries as fracture, rickets, hyperparathyroidism, and so on. The same tumor attacking bone at these different sites should show a similar gradient, structurally and functionally. Homology, architecture, and the indicated differences in their structure and behavior indicate that osteogenic sarcoma, giant cell tumor and osteoid osteoma are genetically identical. Chondromas are derived from the epiphyseal plate. The neoplastic nature of diaphyseal aclasis is rejected.

THE TIME FACTOR IN THE IRRADIATION OF MALIGNANT TUMORS. Perry J. Melnick and (by invitation) Albert Bachem, Chicago, Ill.

Abstract. The purpose of this experiment was to study the histology of tumors irradiated by divided dose methods, as well as to compare the various X-ray treatment methods under controlled conditions. One hundred thirty-one transplantable rat tumors were treated by the massive, fractional, modified protracted-fractional and saturation methods. The results of greatest interest are the demonstration of two different types of degenerative changes in irradiated tumor cells. One of these is well known — the primary necrosis seen in sensitive cells consisting of a characteristic pyknosis and karyorrhexis of the nucleus. The other type of change is seen in refractory tumor cells irradiated by divided dose methods. It consists of the alteration of the nuclei of the cells so that they are transformed into abnormal forms which fail to survive, a kind of lethal mutation. The refractory cells in some of these tumors were transformed, under divided dose methods of irradiation, into practically pure cultures of hyperchromatic giant cells which degenerated in a specific manner by calcification of their nuclei.

Discussion

(Dr. W. C. Hueper, Wilmington.) Several years ago I studied the mineral content and distribution of tumor tissue in incinerated sections prepared from fresh unfixed tissue. It was noticed at that time that tumor cells in the early stages of degeneration showed an increased content of calcium of the nuclei (central calcification). I wonder therefore whether the changes just reported can be considered as specific or whether they represent a phase of the degenerative process commonly found.

(Dr. Shields Warren, Boston.) In the material which Dr. Brues reported the day before yesterday with the use of an agent which injures the nuclei, colchicine, one gets a failure of mitosis to carry through an organized separation of the chromosomes, and frequently individual chromosomes form minute multiple nuclei within the cell which subsequently fuse and form nuclei bizarre in shape and in form, rather resembling the nuclei Dr. Melnick has shown. I should like to inquire whether in the early stages of these processes such scattered nuclei appear.

(Dr. Melnick.) I was able to follow various stages of calcification from the very earliest to the far advanced which you saw on the slide. It is my impression that this is probably a specific degenerative change, although I cannot prove it.

As far as fusion of nuclei is concerned, I saw multinucleated giant cells apparently arising from fusion of several tumor cells, especially in the tumors treated by the massive dose technic; apparently a surface tension change. In these mononuclear giant cells I saw no clear-cut evidence of fusion of multiple nuclei. They divide and we see enormous bizarre mitotic figures, which seem to be individual units.

THE RELATION OF CHRONIC MASTITIS TO CARCINOMA OF THE BREAST. Shields Warren and (by invitation) J. R. E. Morgan and John Fallon, Boston, Mass.

Abstract. To determine the possible precancerous character of breast lesions, we have studied the end results of 783 cases of so-called chronic mastitis and chronic cystic mastitis. Some cases were operated on in Boston and some in Toronto. Five hundred and forty-nine had only excisional biopsy done; the remainder had simple unilateral mastectomy. The follow-up period ranged from 5 to over 20 years, and averaged over 8 years.

Forty-seven cases had recurrence of non-neoplastic breast disease, either in the same, the opposite, or both breasts. Twenty-four cases of breast carcinoma developed, in 3 cases so soon that probably the biopsy failed to demonstrate an already existing carcinoma.

We have calculated the number of cases of breast carcinoma that would be expected in this group, based on the Massachusetts female population of 1930. Several methods of estimating the expectancy were used, and under 4 cases of breast carcinoma should develop in the group during the follow-up period. The actual number (21) is sufficiently greater than the expected to indicate breast disease as a predisposing factor to carcinoma.

As yet criteria are unsatisfactory for determining the lesions most likely to become carcinomatous. Unilateral mastectomy does not protect. Since less than 2.5 per cent of females in this group have developed carcinoma, careful checking for early clinical evidence of malignancy would seem more rational as a prophylactic measure than bilateral mastectomy.

THE RELATION OF MULTIPLE MENINGEAL TUMORS TO VON RECKLINGHAUSEN'S NEUROFIBROMATOSIS. Percival Bailey, Chicago, Ill.

Abstract. A study has been made of two girls, 15 years of age at the time of death, who suffered from multiple tumors of the nervous system. One girl had generalized subcutaneous and cutaneous manifestations of neurofibromatosis. She died following an attempt to remove a tumor from the cerebellopontine angle. At autopsy there were neurofibromas on nearly all the roots of the spinal nerves, a neurofibroma of the twelfth nerve, a glioma of the optic chiasm, a glioma of the cerebellum and nodules of neuroglia cells in the cerebellar cortex; also all the nerves of the body were enlarged and showed the findings of the interstitial hypertrophic neuritis of Dejerine-Sottas. The other girl died following the attempt to remove a tumor from the cerebellar region. At autopsy were found multiple neurofibromas of the roots of the spinal nerves, of the cranial nerves, and interstitial hypertrophic neuritis of the nerve roots, also multiple meningeal tumors, both intracranial and intraspinal, and in addition the typical findings of tuberous sclerosis in the cerebral cortex. The essential relation of the leptomeninges to the other covering cells of the nervous system is again confirmed by these cases.

MULTIPLE TUMORS OF THE SYMPATHETIC NERVOUS SYSTEM WITH A REPORT OF A CASE ILLUSTRATING BOTH BENIGN AND MALIGNANT TYPES. H. R. Wahl and (by invitation) P. E. Craig, Kansas City, Kan.

Abstract. Tumors of the sympathetic nervous system are not common, and may be undifferentiated in structure and malignant in action, or may be be-

nign and well differentiated growths. The latter may be either a ganglioneuroma composed of ganglion cells and nerve fibers, or a paraganglioma, usually arising in the medulla of the adrenal glands and made up of chromaffin cells. Multiple tumors may occur but are very rare. The case reported is that of a young adult negro with a retroperitoneal neuroblastoma combined with two separate retroperitoneal ganglioneuromas, one of which showed extensive secondary hemorrhage and calcification and contained clusters of undifferentiated nerve cells or neurocytes.

Discussion

(Dr. Louise H. Meeker, New York City.) At a recent autopsy on a female 75 years of age an unexpected finding within the duodenum was a mucosal polyp about the size of the thumb; the enlarged polypoid end contained a tumor beneath the mucosa which we have called a paraganglioma, and in that diagnosis Dr. Ewing has concurred.

(Dr. Frederic Parker, Jr., Boston.) I should like to ask what type of tumor metastasized to the vertebrae.

(Dr. Norbert Enzer, Milwaukee.) Were the adrenal glands involved?

(Dr. Wahl.) The adrenal glands were not involved.

The metastases to the vertebrae were apparently of the neuroblastoma type.

EPITHELIAL METAPLASIA OF THE THYROID WITH SPECIAL REFERENCE TO THE HISTOGENESIS OF SQUAMOUS CELL CARCINOMA OF THE THYROID. R. H. Jaffe, Chicago, Ill.

Abstract. In 3 cases of sclerosis of the thyroid and near a metastatic abscess to the thyroid complicating a malignant endocarditis, metaplasia of the epithelium of the follicles to squamous epithelium was observed. This metaplasia resulted in the formation of islands of squamous epithelial cells which occasionally revealed epithelial fibrils. The islands were surrounded by argentaffine fibrils similar to the follicles. It is suggested that epithelial metaplasia may be the source of the rare squamous cell carcinomas of the thyroid, and it is therefore not necessary to trace this type of tumor to embryonic structures, such as the branchial clefts, thyroglossal duct or ultimobranchial bodies. In 1 of 3 cases of squamous cell carcinoma of the thyroid, epithelial metaplasia was found in follicles, in addition to the tumor.

Discussion

(Dr. Stanley Reimann, Philadelphia.) If that is so for the thyroid gland, is it not also true for organs with occasional squamous epithelial lining, such as the gall-bladder, or the urinary tract, or other places where ordinarily there are tall cylindrical cells? In other words, the potentiality of undifferentiated cells — I call them "spare parts" — is at least two; they can turn into tall cells, or into squamous cells. The environment determines which of the two potencies is to be expressed. In Dr. Jaffe's case an abscess determined squamous cells. If you feed an animal a diet deficient in vitamin A, the lining of the trachea turns into squamous epithelium. I am afraid Cohnheim's theory is rapidly becoming of historical interest.

(Dr. Kornel Terplan, Buffalo.) I saw in very rare instances squamous cell

metaplasia in the thyroid. I recall 1 case following severe iodine treatment for Graves' disease in which the surgically removed thyroid showed in addition to typical changes of Graves' disease a very severe interstitial thyroiditis, islands of squamous cells and attempts at keratinization. There were also structures resembling the well known pseudotubercles, made up of so-called epithelioid cells and giant cells of unquestionably epithelial origin.

I should like to ask Dr. Jaffe whether retention or stagnation of colloid in these sclerosing conditions may induce the metaplastic change of the epithelium in the follicles of the thyroid.

(Dr. Shields Warren, Boston.) I should like to ask what the approximate frequency of these changes may be. They have been comparatively rare in my material, I should say less than ten instances among perhaps 12,000 thyroids, and I should also like to ask whether there is any one type of lesion with which they are more apt to occur. In a great many thyroids showing the so-called foreign body reaction to colloid, I have seen them very rarely. On the other hand, where there has been an inflammatory process, such as an abscess, as Dr. Jaffe described, I have the impression they are a bit more frequent.

(Dr. Jaffe.) As far as Dr. Reimann's discussion is concerned, metaplasia may occur in any epithelium wherever tissue breaks down and tissue regenerates and the proliferating cell meets with changed environmental conditions. Not so long ago a case of squamous cell carcinoma of the head of the pancreas came under my observation in which the tumor had developed from the metaplastic epithelium of the pancreatic duct.

Concerning the retention of the colloid, I do not think it is related to this condition, because the follicles first lose their colloid content, then shrink, while some of the epithelial cells degenerate and others proliferate and gradually assume the appearance of squamous cells.

As far as the incidence is concerned I cannot give you an exact figure, but the cases I have presented were found among several thousands of thyroids examined microscopically.

Dr. Warren is right — metaplasia of the follicular epithelium occurs chiefly in inflammation.

The tubercle-like lesions are entirely different. I described them a number of years ago. They are not composed of squamous epithelial cells but of epithelioid cells which are derived from the follicular epithelium.

HISTOPATHOLOGY OF MIXED TUMORS OF THE SALIVARY GLANDS. John W. Budd, Los Angeles, Calif.

Abstract. This study is concerned with the various histological patterns observed in a series of salivary gland tumors. It is believed that the entire group forms a continuity with the following factors responsible for the unusual structures: (*a*) degree and direction of differentiation of the epithelioblast; (*b*) chemical nature of the secretion; and (*c*) direction of the secretion.

Discussion

(Dr. W. C. MacCarty, Rochester, Minn.) Having been something of a technical purist all my life, my esthetic as well as scientific sense has often been offended in these meetings. I have been attending them for nearly 30

years, and the microscopic material which I have seen thrown on the screen has been terrible. Therefore I want now to compliment this young man (I do not know him), on showing the most beautiful histological and cytological pictures I have seen at these meetings. I hope he will keep it up and not fall back into some of the old-fashioned methods of histological and cytological studies.

(Dr. W. S. Hastings, Philadelphia.) I am much interested in Dr. Budd's belief, if I understand him correctly, that this material in the stroma is a secretion from the epithelial cells. In certain instances, at least, I have been strongly inclined to the same view. I should like to ask him, however, how he accounts for the frequent occurrence of cartilage in these tumors.

(Dr. Budd.) I would refer Dr. Hastings to an article by Masson, who can explain the cartilage formation in these tumors far better than I can, but it is merely an accentuation of the process which I attempted to represent.

HISTOLOGICAL STUDIES OF TUMOR REACTIVITY TO BACTERIAL FILTRATES. Isadore E. Gerber and (by invitation) Alice I. Bernheim, New York City.

Abstract. Mice bearing Sarcoma 180 were given a single intravenous injection of 20 to 100 reacting units of meningococcus agar-washings filtrate. The tumors were examined grossly and microscopically with a view to understanding the mechanism underlying the tumor response. The filtrate employed was capable of eliciting the Shwartzman phenomenon in rabbits. One hundred and thirty mice were treated from 3 to 12 days after tumor inoculation, the majority at 10 days, and observed from 4 hours to 10 days after treatment. This series includes only animals surviving the injection of the filtrate.

Six day or older tumors all responded in varying degrees dependent upon the amount of filtrate used. There were severe degenerative changes, striking vascular engorgement, edema and necrosis of the tumor cells followed by either complete regression or regrowth of the tumor from surviving cells. Hemorrhage was seen in the tumor only in the early period (4 hours) following filtrate administration. Lesions of the vessel walls or thrombi were not noted. The effect of the filtrate appeared to be directly on the tumor cells.

Many experimental observations, including our own, suggest that the reaction of the tumor described is analogous to, and is elicited by, the bacterial active principles of the Shwartzman phenomenon. Since no preparatory local injection is required, and since a single intravenous injection is sufficient to call forth the reaction, it appears that the growing neoplastic tissue spontaneously acquires a state of vulnerability.

Discussion

(Dr. M. J. Shear, Boston.) In a good many respects the results obtained in our laboratory are in agreement with those Dr. Gerber has reported. I may add that primary sarcomas produced in mice by hydrocarbons are also susceptible to the action of such filtrates. Our more recent results are in agreement with what Dr. Gerber has just said about the similarities between the hemorrhage in mouse tumors and the Shwartzman phenomenon. Filtrates obtained from *B. coli* were fractionated, and the agent that produced hemorrhage in mouse tumors was separated from accompanying impurities and a highly potent fraction obtained. At this point the material that produced

hemorrhage in mouse tumors in doses of a fraction of a millionth of a gram was then injected into rabbits; it was found that this same material produced hemorrhages in the skin of rabbits, *i.e.* the Shwartzman phenomenon, in doses of the same order of magnitude. I should like to ask Dr. Gerber whether it is true that mice are not susceptible to the Shwartzman phenomenon, as are rabbits, and if that is so, whether he has any explanation as to why mouse tumors should be susceptible.

(Dr. Gerber.) I can answer only the first part of Dr. Shear's question. Mice do not show the Shwartzman phenomenon, as originally defined by Shwartzman. Why the mouse tumor should show it is impossible to answer at the present time.

BOECK'S DISEASE. POSTMORTEM FINDINGS IN A CASE WITH VISCERAL LESIONS. Max Pinner, Ithaca, N. Y.

Abstract. The autopsy findings in a case of Boeck's sarcoid are presented. The characteristic granulomas, consisting of miliary nodules of epithelioid cells without, or with a paucity of other tissue changes, were present in the skin, many lymph nodes, lungs, pericardium, kidneys and spleen. In this particular case the potential developments of granuloma can be demonstrated in a rather convincing manner. They are (1) a singularly coarse hyalinized fibrosis, and (2) caseation — that is, transformation into banal tuberculosis which, however, is distinguished by practically complete absence of exudative features. Hyaline fibrotic nodules, evidently late stages of epithelioid granuloma, were found in all organs mentioned except the skin, and in the bone marrow of phalanges and sternum, causing in the former the characteristic roentgenological appearance of osteitis tuberculosa cystoides multiplex (Jüngling). Caseated nodules were found in all organs mentioned and in the myocardium, but not in the skin. In the lungs the process led to a diffuse, hyaline interstitial fibrosis. A study of this case, supported by considerable evidence from the literature, leaves little doubt that Boeck's sarcoid is a particular form of tuberculosis.

Discussion

(Dr. Ralph D. Lillie, Washington, D. C.) Some of the cases of Boeck's disease in which we have seen the cutaneous manifestations were definite cases of maculoanesthetic or nerve leprosy. In hearing Dr. Pinner's presentation I wondered whether the caseation, and the appearance of acid-fast bacilli, and the cavitation of the lungs were a part of the Boeck's disease, or the supervening of an active tuberculosis on a possibly leprous lesion, a thing that is a very common finding in leprosy when it is institutionalized.

(Dr. Henry C. Sweany, Chicago.) I have been very much interested in this presentation because it is near to my heart. Many years ago we had an experience with certain types of tubercle bacilli that were not what we would call typical, and I believe that pathologists in general should look to the variations in the tubercle bacillus for variations in pathology more than they usually do. Pathologists have attempted to explain much variation in pathology on the basis of changes in the host and constitution, dosage, allergy, and so on, but I do not believe there has been enough attention paid to these rare occasional offshoots, which we may call variations at certain times and, if we

choose, mutations at others. Smithburn, for example, has driven a very fine wedge into this problem by showing that we can vary almost any strain of tubercle bacillus by varying the hydrogen ion concentration, and these variations produce variations in pathology, according to the variation in the bacillus. I believe that many of these variants do produce pathological changes that parallel the variation. I want to ask Dr. Pinner, however, if he cultured these lesions, if he obtained growths from the culture or animal inoculation and, if so, what was the characteristic of the growth?

(Dr. Frederic Parker, Jr., Boston.) As I understood Dr. Pinner, he said there was caseation present. In making the diagnosis of the cases of Boeck's disease I have seen, we thought the presence of caseation automatically ruled out the diagnosis of sarcoid. I should like to ask Dr. Pinner concerning this point.

(Dr. Pinner.) I think I can say very definitely that this case is not leprosy. The skin lesions were quite slight and there was no clinical evidence even to suggest leprosy. I am familiar with the cystic bone lesions in leprosy that have a similar appearance to the ones shown in this case, but this is the only similarity between leprosy and the case presented.

As far as the bacteriology is concerned, I was afraid that would come up. We did make cultures and we did not get any growth from the lymph nodes. We did not make animal inoculations because our laboratory at the time was in a very embryonic state and we were unable to do it for technical reasons.

As far as the question of caseation is concerned, I am perfectly willing to agree that caseation should exclude the diagnosis of Boeck's disease. The skin lesions and the lymph node lesions which I showed at first were characteristic of Boeck's disease and did not show any caseation. As a matter of fact, the first two slides, the one from the skin and the first lymph node lesion, were biopsy material on this patient. On that basis we felt justified in making a diagnosis of Boeck's disease. When caseation supervenes, I think, it is entirely a question of taste whether one wishes to say that now Boeck's disease has become tuberculosis, or whether one chooses to say that caseation is a potential development of Boeck's disease. In my opinion, not based only on this case but on an analysis of the literature as well, the entire terminology of Boeck's disease, or benign miliary lupoid, is entirely unnecessary. All we have to say is that so-called Boeck's disease is a particular type or phase of tuberculosis, which, similar to any other form of tuberculosis, has the two potential developments, fibrosis and caseation, as demonstrated in this case.

Going back to Dr. Sweany's discussion, in spite of the fact that we were not able to isolate the organism, I think the evidence is definitely in favor of assuming that the particular type of lesion characterized as Boeck's disease is not due to any variation in the bacillus, but to an intrinsic factor, because a very large percentage of patients with these lesions are anergic to tuberculin. So was this patient until 4 weeks before his death. Up to that time his sputum on many occasions was negative. Four weeks before death he showed tubercle bacilli in the sputum and tuberculin allergy. Tuberculin anergy and absence of caseation seem to be closely associated; which is the cause and which the effect we do not know, but there is undoubtedly a close relation between caseation and tuberculin allergy.

THE EFFECTS OF COAL SMOKE OF KNOWN COMPOSITION ON RABBIT LUNGS.
Lucy Schnurer (by invitation) and Samuel R. Haythorn, Pittsburgh, Pa.

Abstract. Bituminous coal was burned continuously in an egg stove over a period of 80 days and about 10 per cent of the flue smoke diverted, mixed with oxygen and passed through air-tight chambers containing animals. Dust counts and analyses for gas contents were made daily. The smoke particles were 75 per cent carbon, and the ash contained 1.1 per cent silica particles by count. Some of the animals were killed at the end of the exposure and the rest were returned to animal house conditions to be examined at varying periods. The last set was examined over a year after the exposure had ceased. Immediately after exposure the lungs contained great quantities of carbon phagocytes and much free carbon. Later the phagocytes began to be collected in clumps in the alveoli and in various sets of lymphatics. The final animals showed many nodular collections surrounded by areas of pneumonitis and interspersed with collagen fibrils. The pigmented phagocytes in the lymph nodes first examined were diffuse. In the later nodes they were gathered in large confluent masses. The lung changes resembled those of persons living in smoky atmospheres rather than those associated with occupational diseases. The results indicated that carbon in sufficient quantities is capable of producing fibrous changes. A way was found to prepare the lungs of rabbits for further studies involving the relation of smoke and pulmonary infections.

Discussion

(Dr. Arthur J. Vorwald, Saranac Lake.) What was the method of making the dust counts? Was it by light or by dark field?

(Dr. Haythorn.) I did not make the dust counts. They were made by light field at 970 diameters. Another thing I neglected to say was that we did incinerations of all these sections and could find no crystalline substance in any of the incinerated residues.

(Dr. Vorwald.) Is it possible that substances present in the smoke, other than the dust *per se*, might have been responsible for the pulmonary fibrosis observed in the experimental animals? I am referring particularly to the gases that were liberated and subsequently inhaled by this method of experiment.

(Dr. Haythorn.) The only chemical analysis I have here showed no other substances except those that were given, namely oxygen, carbon monoxide, carbon dioxide, hydrogen sulphide, and sulphur dioxide, and the dust particles. We do not know what was in the ash fraction. There were several amorphous things there not identified, and it was not impossible that there were some other substances. However, this kind of smoke is the sort of thing we go around breathing all the time and we find that it does not increase the incidence of tuberculosis. The lung picture is the same that we have found associated with much organizing pneumonia in our district.

READ BY TITLE

THE QUARTZ CONTENT OF SILICOTIC LUNGS DETERMINED BY THE X-RAY DIFFRACTION METHOD. Dudley A. Irwin, Toronto, Canada.

THE METEOROLOGICAL INFLUENCE ON THE OCCURRENCE OF HEMORRHAGE AND PERFORATION IN PEPTIC ULCER. George Milles (by invitation), Chicago, Ill.

ETIOLOGICAL FACTORS OF APPENDICITIS MORTALITY. Frederick W. Mulsow, Cedar Rapids, Ia.

THE COMPARATIVE THICKNESS OF NORMAL, SULFHYDRAL-TREATED, AND DISULFOXIDE-TREATED MOUSE SKINS. Stanley P. Reimann, Philadelphia, Pa.

HISTOLOGICAL STUDIES OF A MUSCLE HEMANGIOMA. George Rukstinat, Chicago, Ill.

MORPHOLOGICAL VARIATIONS OF TUMOR CELLS. Otto Saphir, Chicago, Ill.

COMPLETE OCCLUSION OF THE ENTIRE VENA CAVA BELOW THE HEPATIC VEINS. A. L. Sparks (by invitation), Cleveland, Ohio, and Herbert Fox, Philadelphia, Pa.

THE PATHOLOGY OF OMENTAL CYSTS. F. W. Wiglesworth, Montreal, Canada.

VASCULARITY OF THE BLOOD VESSEL WALL. ADAPTIVE CHANGES. Milton C. Winternitz and (by invitation) Robert M. Thomas, New Haven, Conn.